

2014

# Gender Differences in High Sensitivity C - Reactive Protein and Self-Reported Muscle Strengthening Activity Among U.S. Adults

Michael R. Richardson
*University of North Florida*



# GENDER DIFFERENCES IN HIGH SENSITIVITY C - REACTIVE PROTEIN AND SELF-REPORTED MUSCLE STRENGTHENING ACTIVITY AMONG U.S. ADULTS

by

Michael Ryan Richardson

A thesis submitted to the Department of Clinical and Applied Movement Science

in partial fulfillment of the requirements for the degree of

Master of Science in Exercise Science and Chronic Disease

UNIVERSITY OF NORTH FLORIDA

BROOKS COLLEGE OF HEALTH

April, 2014

The thesis of Michael Ryan Richardson is approved: (Date)

_____ _____

Dr. James R. Churilla, Ph.D., M.P.H., M.S., RCEP, FACSM
Committee Chairperson

_____ _____

Dr. Tammie M. Johnson, Dr.P.H., M.P.H.
Committee Member

_____ _____

Dr. Earl S. Ford, M.D., M.P.H.
Committee Member

_____ _____

Dr. Peter T. Katzmarzyk, Ph.D., FACSM, FAHA
Committee Member

Accepted for the Department:

_____ _____

Dr. Joel W. Beam, Ed.D, ATC, LAT
Chair, Department of Clinical & Applied Movement Sciences

Accepted for the College:

_____ _____

Dr. Pamela S. Chally, PhD, RN
Dean, Brooks College of Health

Accepted for the University:

_____ _____

Dr. Len Roberson, PhD
Dean of the Graduate School

## Dedication & Acknowledgements

The completion of a Master's program requires a strong commitment and a great amount of support. My academic goals have become a reality because of the patience and support of my family. This thesis project is dedicated to my wife and children. You will always be a source of inspiration to me.

Thank you to my wife Kim Richardson for her love, inspiration and encouragement. Without your support this project would have never been possible. Also, thank you to my girls Alora and Avery for their patience, understanding, and sacrifices to make this project a possibility.

I would also like to thank the Clinical and Applied Movement Sciences faculty and my committee members Dr. Tammie M. Johnson, DrPH, M.P.H., Dr. Earl S. Ford, M.D., M.P.H., and Peter T. Katzmarzyk, Ph.D., FACSM, FAHA for their guidance and leadership. You exemplify research excellence. Thank you also goes to my fellow graduate research assistant William R. Boyer, who without his assistance and friendship this project would not have been possible.

Finally, I would like to thank my committee chairperson and mentor, Dr. James R. Churilla, Ph.D., MPH, M.S., RCEP, FACSM, for his honesty, candor, and friendship. You have been an endless source of support. You led by example and continually encouraged me to reach higher toward new goals and to expect great things.

## Table of Contents

**Appendices**

## List of Tables

**Table** **Page**

### Chapter One: Introduction



### Chapter Two: Review of Literature



### Chapter Four: Gender Differences in High Sensitivity C - reactive Protein and Self-Reported Muscle Strengthening Activity among U.S. Adults

# List of Figures

**Figure** **Page**

## Nomenclature

| | |
|---|---|
| cm | centimeter |
| d/wk | days per week |
| $kg/m^2$ | kilograms per meter squared |
| $MET \cdot min \cdot wk^{-1}$ | MET minutes per week |
| mg/dL | milligrams per deciliter |
| mg/L | milligrams per liter |
| min/wk | minutes per week |
| mmHg | millimeters of mercury |
| mo | month |
| ng/mL | nanograms per liter |
| times/mo | times per month |
| time(s)/wk | time(s) per week |
| yr | year |

## List of Abbreviations

| | |
|---|---|
| AA | African American |
| ACLS | Aerobics Center Longitudinal Study |
| AHA | American Heart Association |
| AT | aerobic training |
| BMI | body mass index |
| CDC | Centers for Disease Control and Prevention |
| CG | control group |
| CHD | coronary heart disease |
| CI | confidence interval |
| CRP | C - reactive protein |
| CT | concurrent training |
| CV | cardiovascular |
| CVD | cardiovascular disease |
| Da | Dalton |
| DBP | diastolic blood pressure |
| DHHS | Department of Health and Human Services |
| DXA | dual-energy x-ray absorptiometry |
| ELSA | English Longitudinal Study of Ageing |
| eNOS | endothelial nitric oxide synthase |
| HART-D | Health Benefits of Aerobic and Resistance Training in individuals with type 2 diabetes |
| HDL-C | high-density lipoprotein cholesterol |

| | |
|---|---|
| HIV | human immunodeficiency virus |
| hs | high sensitivity |
| IL-1 | interleukin-1 |
| IL-6 | interleukin-6 |
| KORA | Kooperative Gesundheitsforschung in der Region Augsburg |
| LDL-C | low density lipoprotein cholesterol |
| LTPA | leisure-time physical activity |
| LTSB | leisure-time sedentary behavior |
| MCQ | medical conditions questionnaire |
| MEC | mobile examination center |
| MET | metabolic equivalent |
| MI | myocardial infarction |
| MIPA | moderate intensity physical activity |
| MIS | maximum isometric strength |
| MS | multiple sclerosis |
| MSA | muscle strengthening activity |
| NCEP ATP III | National Cholesterol Education Panel Adult Treatment Panel III |
| nH | non-Hispanic |
| NHANES | National Health and Nutritional Examination Survey |
| NHLBI | National Heart, Lung, and Blood Institute |
| non-HDL-C | non-high density lipoprotein cholesterol |
| OR | odds ratio |
| PA | physical activity |

| | |
|---|---|
| PAD | peripheral arterial disease |
| PAQ | physical activity questionnaire |
| PCh | phosphocholine |
| PRINCE | Pravastatin Inflammation/CRP Evaluation |
| RM | repetition maximum |
| RT | resistance training |
| SAS | statistical analysis software |
| SBP | systolic blood pressure |
| SE | standard error |
| SP | spouse |
| ST | screen time |
| SUDAAN | software for survey data analysis |
| T2D | type 2 diabetes |
| TC | total cholesterol |
| TNF-α | tumor necrosis factor-alpha |
| VIPA | vigorous intensity physical activity |
| WA | white American |
| WC | waist circumference |
| WTH | waist-to-hip ratio |

## Abstract

**Objectives** We sought to examine the gender differences between C - reactive protein (CRP) and muscle strengthening activity (MSA) in U.S. adults (≥20 years of age).

**Background** Elevated levels of CRP have been shown to be associated with an increase in risk of cardiovascular disease (CVD). Studies analyzing the relationship between physical activity (PA) and CRP by gender have produced mixed results.

**Methods** The sample (n=9,135) included participants in the 1999-2004 National Health and Nutrition Examination Survey (NHANES). Three categories of reported MSA participation were created: no MSA (referent group), some MSA (≥1 to <2 d/wk), and meeting the 2008 Department of Health and Human Services (DHHS) recommendation (≥2 d/wk). The dependent variable was elevated CRP (>3 to 10 mg/L).

**Results** Gender stratified analysis revealed significantly lower odds of having elevated CRP for women reporting some MSA (OR 0.61; 95% CI 0.45-0.83, P=0.0023), or volumes of MSA meeting the DHHS recommendation (OR 0.66; 95% CI 0.54-0.82, P=0.0004). Significantly lower odds of men having elevated CRP was observed in those reporting MSA volumes meeting the recommendation (OR 0.73; 95% CI 0.61-0.88, P=0.0011). Following adjustment for waist circumference (WC) these odds remained significant in men but not women.

**Conclusions** Women reporting any MSA were found to have lower odds of having elevated CRP when compared to those reporting no MSA prior to adjustment for WC. Significantly lower odds in men were only observed in those meeting the recommendation. These results suggest that WC may mediate the associations between MSA and CRP and this relationship may be stronger in women.

# Chapter One: Introduction

C - reactive protein (CRP) is an acute phase protein synthesized by the liver and adipose tissue in response to inflammation (1-4). CRP levels measured by high sensitivity (hs)-CRP assays have shown efficacy in predicting risk of vascular events (5-7). Previous studies have found that CRP levels are associated with cardiorespiratory fitness levels and self-reported physical activity (PA) (8,9). This chapter provides an overview of CRP, discusses the current guidelines for clinical practice, and provides an abbreviated literature review. It concludes with the problem being addressed, the purpose of this research, and the operational definitions for the relevant terminology.

## Background

Elevated levels of CRP, a non-specific marker of inflammation, have been shown to be associated with an increase in risk of cardiovascular disease (CVD) (1). Moreover, previous cross-sectional analyses have revealed significant positive associations between CRP concentration and self-reported myocardial infarction (10) and stroke (11). It has also been shown that PA participation has a role in reducing incidence of CVD (12). Interestingly, though PA may produce a short term rise in circulating levels of CRP, long term participation in exercise training has been shown to reduce CRP levels (13). Some studies have shown that the associations between PA and CVD may be mediated largely by inflammation (14,15).

## Prevalence of Elevated CRP

Using data from NHANES 1999-2000, a nationally representative sample, Ajani et al. (16) found that approximately one in every four persons ≥20 years of age, without

coronary heart disease (CHD) or diabetes, and with serum lipid concentrations within recommended ranges, had a CRP concentration >3 to 10 mg/L (Table 1). The prevalence estimates of high CRP varied with body mass index (BMI), with approximately one-third of obese people, with lipid concentrations within recommended ranges and without CHD or diabetes, having high CRP concentrations.

**Table 1: Prevalence of high CRP concentrations (>3.0 mg/L) among persons with serum lipid concentrations within recommended ranges after exclusion of those with CRP >10 mg/L, NHANES 1999–2000**

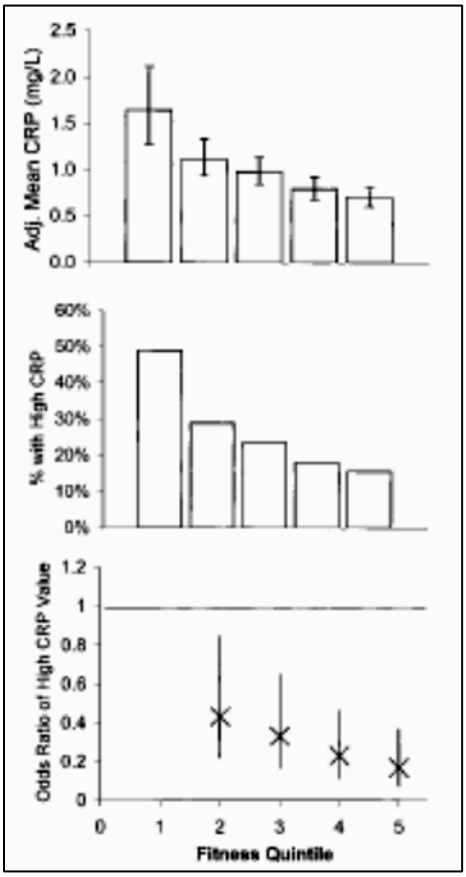
| | Normal LDL-C[1] | Normal HDL-C[1] | Normal TC[1] | Normal lipid profile[1] |
|---|---|---|---|---|
| All participants | | | | |
| Weighted percentage with concentrations within recommended ranges | 57.4% | 75.9% | 51.4% | 34.2% |
| Median CRP concentrations, mg/L | 1.7 | 1.6 | 1.4 | 1.2 |
| Crude (SE) prevalence of high CRP, % | 26.7 (1.9) | 28.1 (2.2) | 24.3 (1.9) | 22.2 (2.4) |
| Adjusted (SE)[2] prevalence of high CRP, % | 27.3 (1.9) | 27.3 (2.2) | 25.8 (2.0) | 22.9 (2.5) |
| Estimated no. with high CRP | 26 900 632 | 37 538 753 | 21 915 520 | 13 363 660 |
| Persons without CHD and diabetes mellitus | | | | |
| Weighted percentage with concentrations within recommended ranges | 57.1% | 77.2% | 51.3% | 35.1% |
| Median CRP concentrations, mg/L | 1.7 | 1.6 | 1.4 | 1.1 |
| Crude (SE) prevalence of high CRP, % | 26.2 (2.0) | 27.5 (2.2) | 24.0 (2.1) | 22.1 (2.7) |
| Adjusted (SE)[2] prevalence of high CRP, % | 27.1 (2.0) | 26.6 (2.2) | 25.9 (2.2) | 23.1 (2.7) |
| Estimated no. with high CRP | 23 577 949 | 33 359 076 | 19 336 181 | 12 243 745 |
| Stratified by body mass index | | | | |
| Normal (<25.0 kg/m$^2$) | | | | |
| Crude (SE) prevalence of high CRP, % | 15.9 (2.4) | 16.0 (2.3) | 12.7 (2.5) | 13.0 (2.7) |
| Adjusted (SE)[2] prevalence of high CRP, % | 17.2 (2.2) | 16.0 (2.2) | 14.7 (2.5) | 14.4 (2.7) |
| Estimated no. with high CRP | 6 980 415 | 9 474 718 | 5 067 842 | 4 060 391 |
| Overweight (25.1 to <30.0 kg/m$^2$) | | | | |
| Crude (SE) prevalence of high CRP, % | 30.3 (3.8) | 30.9 (3.3) | 28.4 (4.0) | 30.9 (5.8) |
| Adjusted (SE)[2] prevalence of high CRP, % | 30.9 (4.1) | 29.8 (3.4) | 30.3 (4.3) | 31.6 (5.8) |
| Estimated no. with high CRP | 8 291 472 | 11 480 752 | 7 070 541 | 4 619 161 |
| Obese (≥30 kg/m$^2$) | | | | |
| Crude (SE) prevalence of high CRP, % | 44.6 (4.3) | 49.7 (4.0) | 44.9 (4.7) | 38.9 (5.8) |
| Adjusted (SE)[2] prevalence of high CRP, % | 43.0 (3.9) | 46.1 (4.1) | 44.1 (4.3) | 36.0 (5.1) |
| Estimated no. with high CRP | 8 306 062 | 12 403 606 | 7 197 798 | 3 564 194 |

*Note*. Adapted from "Prevalence of high C - reactive protein in persons with serum lipid concentrations within recommended values" by Ajani et al. *Clin Chem* 50(9):1618-22, 2004. Copyright 2014 by the American Association for Clinical Chemistry.

In a study investigating associations between cardiorespiratory fitness levels and elevated CRP (≥2 mg/L), Church et al. (8) found fitness levels to be inversely associated with elevated CRP values in men participating in the Aerobics Center Longitudinal Study

(ACLS), independent of BMI (figure 1). These investigators revealed a significant inverse trend in the prevalence of elevated CRP across fitness quintiles (P<0.0001). In comparison to men in the lowest fitness quintile, all other fitness quintiles had significantly lower odds of having elevated CRP, with the lowest odds of high CRP being found in the highest fitness category, odds ratio (odds ratio [OR] 0.17, 95% confidence interval [CI] 0.08 - 0.37; P=0.001). The OR for men having elevated CRP in the lowest fitness quintile compared with all other quintiles was 3.2 (95% CI 1.8 - 5.8; P<0.0001). The prevalence of elevated CRP across fitness quintiles, low to high, was 48%, 27%, 24%, 22%, and 15% (P for trend <0.0001).

Figure 1: Data from 722 men in the ACLS. Data were adjusted for age, BMI, vitamin use, statin medication use, aspirin use, the presence of inflammatory disease, CVD, diabetes, and smoking. The top panel presents adjusted geometric mean CRP values across fitness quintiles, the middle panel depicts the percentage of individuals with a high CRP (≥2.0 mg/L) across fitness quintiles, and the bottom panel presents the odds of having high CRP across fitness quintiles, with the lowest quintile as the referent group. Error bars represent 95% CIs.




*Note*. Adapted from "Associations between cardiorespiratory fitness and C - reactive protein in men" by Church et al. *Arterioscler Thromb Vasc Biol* 22(11):1869-76, 2002. Copyright 2002 by the American Heart Association.

In a study using data from 15,341 male and female participants in NHANES III, Miller et al. (17) investigated the prevalence of high CRP using CHD risk factor cut points. Investigators in this study reported an overall prevalence of 25.7% for high CRP. Results of this study showed higher levels of CRP for blacks as well as markedly higher prevalence's of high CRP with higher blood pressure, glucose levels, and BMI. Also, high CRP levels were more common in both former and active cigarette smokers when compared to those who had never smoked.

In comparison to a non-smoker referent group with no CHD risk factors, the presence of at least one borderline or abnormal CHD risk factor was associated with approximately three fold higher prevalence of CRP levels >3 mg/L. The prevalence of elevated CRP was also higher among postmenopausal women receiving hormone replacement therapy when compared to nonusers and former users, 51% versus 37%, respectively. Moderate and/or vigorous PA was not included in analysis. The risk of elevated CRP levels attributable to the presence at least one abnormal or borderline CHD risk factor was found to be approximately 78% for men and 67% for women (17).

**Current Clinical Practice Recommendations**

In a statement issued for healthcare professionals from the Centers for Disease Control and Prevention (CDC) and the American Heart Association (AHA) (18), clinical applications and health practice questions regarding CRP assay use and risk level cut points are addressed. Standard CRP assays have lower detection limits of 3-8 mg/L. Since these assays lacked the sensitivity to accurately measure CRP ranges within the low to normal range, they could not be employed in cardiovascular risk prediction research. This limitation prompted epidemiological studies to begin using assays designed to determine CRP levels across the normal range. These assays are referred to as "high-sensitivity" (hs) or "ultra-sensitive" assays, which are now commercially available (19).

Thus, the use of the hs-CRP assay for CRP measurement is currently recommended. This recommendation is due to hs-CRP analyte precision, accuracy, and availability. The hs-CRP assay should only be performed on apparently healthy people

not displaying signs of acute inflammation and assay results should be expressed as milligrams per liter (mg/L). The average of two assays, two weeks apart, should be used to provide the most stable CRP estimate; a fasting state is not required. Lastly, since CRP levels of 10 to 40 mg/L are associated with mild inflammation, and levels of 40 to 200 mg/L with an acute inflammation and bacterial infection, CRP levels >10 mg/L should be considered due to acute inflammation and discarded from analysis (6,7,18,20).

The recommended CRP level cut points for clinical use (Table 2) are <1.0 mg/L for low risk, 1.0-3.0 mg/L for average risk, and >3.0 mg/L for high risk, with the high risk tertile cited as having approximately two-fold increase in relative risk of CVD when compared with the low tertile (18). These tertiles were based on the distributions of hs-CRP from >15 populations, >40 000 people, collected for the purpose of defining the population distribution. Largely, the high risk hs-CRP category included the skewed tail of the distribution (18). The cost of CRP screening has been compared to that of standard cholesterol testing and by adding CRP to LDL-C screening there stands the potential for an immediate cost savings and circumvention of additional and unnecessary testing (6).

**Table 2: CDC and the AHA Recommendations for Use of Inflammatory Markers in Clinical and Public Health Practice**

| Cardiovascular Risk Level | Laboratory Value (hs-CRP, mg/L) |
|---|---|
| Low | <1 |
| Average | 1-3 |
| High | >3 |

*Note*. Adapted from "Markers of Inflammation and Cardiovascular Disease Application to Clinical and Public Health Practice; A Statement for Healthcare Professionals from the Centers for Disease Control and Prevention and the American Heart Association" by Pearson et al. *Circulation* 107(3):499-511, 2003. 

## Abbreviated Literature Review

Though some studies have shown that participation in various forms of muscle strengthening activity (MSA) is associated with lower circulating levels of CRP, the

study populations have been small and not representative of the U.S. population (21-23). Moreover, when stratified by gender, studies analyzing the distribution of elevated CRP (>3 mg/L), or the relationship between PA and CRP, have produced mixed results and the implications remain unclear (24-29).

**Purpose and Research Questions**

The purpose of this study was to examine the associations between self-reported MSA and CRP in adult participants of the NHANES when stratified by gender. Emphasis was placed on determining whether volumes of MSA meeting the 2008 Department of Health and Human Services (DHHS) recommendation (30) are associated with significantly lower odds of elevated CRP and examining the mediating effects of central adiposity on this association. The specific research questions addressed by this study are as follows:

1. Is there an association between volumes of MSA meeting the current DHHS recommendation and having an elevated CRP level?
2. If a relationship does exist, is that relationship different for each gender?
3. Does waist circumference (WC) mediate this relationship?

To the extent of our knowledge, this is the first study to examine the potential gender differences in high sensitivity measured CRP levels and volumes of MSA in adults aged ≥20 years in NHANES 1999-2004. Ultimately, this study adds to the evidence demonstrating gender specific associations between MSA and cardio-metabolic risk.

**Project Description**

For this study, the sample was limited to adults (≥20 years of age). Participants who attended a mobile examination center (MEC) in the 1999-2004 NHANES were included in the resultant analyses. Pregnant women were excluded from the analyses. Lastly, the participants included in the analyses had complete data on all the variables of interest. The present study has several inherent limitations due to the design. Consequently, all findings must be interpreted with caution. These limitations are as follows:

1. The most recent NHANES MSA data were collected from 1999-2004. Therefore the analyzed data may not be reflective of the current U.S. adult population.
2. Due to the nature of the cross-sectional study design, causality will not be able to be established.
3. The MSA data was self-reported over the past 30 days; as a result the frequency of MSA is subject to recall bias.
4. The MSA data is not objectively measured

**References**


1. Kaptoge S, Di Angelantonio E, Lowe G, et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet 2010;375:132-40.
2. Black S, Kushner I, Samols D. C-reactive Protein. J Biol Chem 2004;279:48487-90.
3. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? Arterioscler Thromb Vasc Biol 1999;19:972-8.
4. Pepys MB, Berger A. The renaissance of C reactive protein. BMJ 2001;322:4-5.
5. Ridker PM, Silvertown JD. Inflammation, C-reactive protein, and atherothrombosis. J Periodontol 2008;79:1544-51.
6. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation 2003;107:363-9.
7. Jialal I, Devaraj S, Venugopal SK. C-reactive protein: risk marker or mediator in atherothrombosis? Hypertension 2004;44:6-11.
8. Church TS, Barlow CE, Earnest CP, Kampert JB, Priest EL, Blair SN. Associations between cardiorespiratory fitness and C-reactive protein in men. Arterioscler Thromb Vasc Biol 2002;22:1869-76.
9. Ford ES. Does exercise reduce inflammation? Physical activity and C-reactive protein among U.S. adults. Epidemiology 2002;13:561-8.

10. Ford ES, Giles WH. Serum C-reactive protein and fibrinogen concentrations and self-reported angina pectoris and myocardial infarction: findings from National Health and Nutrition Examination Survey III. J Clin Epidemiol 2000;53:95-102.

11. Ford ES, Giles WH. Serum C-reactive protein and self-reported stroke: findings from the Third National Health and Nutrition Examination Survey. Arterioscler Thromb Vasc Biol 2000;20:1052-6.

12. Nocon M, Hiemann T, Muller-Riemenschneider F, Thalau F, Roll S, Willich SN. Association of physical activity with all-cause and cardiovascular mortality: a systematic review and meta-analysis. Eur J Cardiovasc Prev Rehabil 2008;15:239-46.

13. Kasapis C, Thompson PD. The effects of physical activity on serum C-reactive protein and inflammatory markers: a systematic review. J Am Coll Cardiol 2005;45:1563-9.

14. Mora S, Cook N, Buring JE, Ridker PM, Lee IM. Physical activity and reduced risk of cardiovascular events: potential mediating mechanisms. Circulation 2007;116:2110-8.

15. Wannamethee SG, Lowe GD, Whincup PH, Rumley A, Walker M, Lennon L. Physical activity and hemostatic and inflammatory variables in elderly men. Circulation 2002;105:1785-90.

16. Ajani UA, Ford ES, Mokdad AH. Prevalence of high C-reactive protein in persons with serum lipid concentrations within recommended values. Clin Chem 2004;50:1618-22.

17. Miller M, Zhan M, Havas S. High attributable risk of elevated C-reactive protein level to conventional coronary heart disease risk factors: the Third National Health and Nutrition Examination Survey. Arch Intern Med 2005;165:2063-8.

18. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003;107:499-511.

19. Ridker PM. High-sensitivity C-reactive protein: potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. Circulation 2001;103:1813-8.

20. Myers GL, Rifai N, Tracy RP, et al. CDC/AHA Workshop on Markers of Inflammation and Cardiovascular Disease: Application to Clinical and Public Health Practice: report from the laboratory science discussion group. Circulation 2004;110:e545-9.

21. Heffernan KS, Jae SY, Vieira VJ, et al. C-reactive protein and cardiac vagal activity following resistance exercise training in young African-American and white men. Am J Physiol Regul Integr Comp Physiol 2009;296:R1098-105.

22. Stewart LK, Flynn MG, Campbell WW, et al. The influence of exercise training on inflammatory cytokines and C-reactive protein. Med Sci Sports Exerc 2007;39:1714-9.

23. Donges CE, Duffield R, Drinkwater EJ. Effects of resistance or aerobic exercise training on interleukin-6, C-reactive protein, and body composition. Med Sci Sports Exerc 2010;42:304-13.

24. Isasi CR, Deckelbaum RJ, Tracy RP, Starc TJ, Berglund L, Shea S. Physical fitness and C-reactive protein level in children and young adults: the Columbia University BioMarkers Study. Pediatrics 2003;111:332-8.

25. Albert MA, Glynn RJ, Ridker PM. Effect of physical activity on serum C-reactive protein. Am J Cardiol 2004;93:221-5.

26. Canon ME, Crimmins EM. Sex differences in the association between muscle quality, inflammatory markers, and cognitive decline. J Nutr Health Aging 2011;15:695-8.

27. Majka DS, Chang RW, Vu TH, et al. Physical activity and high-sensitivity C-reactive protein: the multi-ethnic study of atherosclerosis. Am J Prev Med 2009;36:56-62.

28. Khera A, McGuire DK, Murphy SA, et al. Race and gender differences in C-reactive protein levels. J Am Coll Cardiol 2005;46:464-9.

29. Geffken DF, Cushman M, Burke GL, Polak JF, Sakkinen PA, Tracy RP. Association between physical activity and markers of inflammation in a healthy elderly population. Am J Epidemiol 2001;153:242-50.

30. Physical activity guidelines for Americans; Be active, healthy, and happy. United States Department of Health and Human Services 2008.

# Chapter Two: Review of Literature

C - reactive protein (CRP) is regarded as a marker of acute phase inflammation and chronic low-grade inflammation and has shown value as a predictor of future risk for cardiovascular disease (CVD) (1,2). The levels described as low grade systemic inflammation have been previously characterized by a two-to-threefold increase in concentrations of CRP (3). Evidence suggests that elevated CRP levels are predictive of myocardial infarction (MI), stroke, peripheral arterial disease (PAD), and sudden cardiac death (4). Risk of CVD has also been shown to increase with increased leisure-time sedentary behavior (LTSB), such as increased screen time, and decrease with participation in vigorous activity. These studies revealed increased mortality and CVD risk regardless of physical activity participation, thus warranting further analysis using population based reports of LTSB and the associations with CRP.

The following literature review will discuss 1) the history, structure, and production of CRP; 2) a brief summary of the relationship between CRP and atherosclerosis; 3) a concise explanation of the relationship between myocytes and CRP production; 4) concise reviews of the previous studies examining the associations between MSA and CRP.

**CRP: Discovery, Structure, and Production**

CRP was discovered in 1930 at the Rockefeller Institute for Medical Research by William S. Tillet and Thomas Francis in the serum of patients with acute inflammation due to *streptococcus pneumonia* infection (5). Serum obtained from these patients during the acute phase response of the infection was found to contain a protein, at first referred to as “fraction C”, that could precipitate the “C” polysaccharide derived from the

pneumococcal cell wall. Following this discovery in 1941, Oswald T. Avery and Theodore J. Abernethy would find that the substance that was responsible for the precipitation with fraction C was a protein, thus dubbed CRP, with calcium being essential to the reaction (6,7).

CRP plays a role in the innate immune system by activating the classical complement pathway. Through the calcium dependent binding of ligands containing phosphocholine, as well to the phospholipids of damaged cells, CRP enhances the uptake of damaged cells by macrophages (8,9). Initially, CRP was considered a pathogenic secretion elevated in people suffering from one of many forms of tissue damage (10). However, synthesis by hepatocytes demonstrates that CRP is a native protein. At the transcriptional level, CRP is regulated by the cytokine interleukin (IL)-6 and enhanced IL-1, with tumor necrosis factor (TNF)-α possibly playing a part in CRP synthesis through influencing IL-6 production (6,11).

CRP is a protein of the pentraxin family. It consists of five non-covalently bound identical protomers arranged around a central pore. Each protomer subunit consists of a single polypeptide chain of 206 amino acids with a total molecular weight of approximately 23,000 Dalton (Da). Each subunit has two calcium ions surrounding a hydrophobic pocket, thus making it capable of forming calcium-dependent bonds with ligands, usually phosphocholine (PCh). The above described Fraction C, present on the bacterial cell wall, contains phosphocholine (6,7). It is a non-glycosylated protein and the gene has been mapped to chromosome 1 (12).

**CRP and Atherosclerosis**

C - reactive protein has been shown to possess several properties that make it capable of increasing atherosclerosis. C - reactive protein has been shown to increase the expression adhesion molecules by endothelial cells. Additionally, CRP has been shown to bind to and enhance the uptake of oxidized low-density lipoprotein cholesterol (LDL-C) by macrophages (6,8), a process which some investigators have concluded enhances foam cell formation (13). Furthermore, CRP has been shown to decrease the bioavailability of endothelial nitric oxide synthase (eNOS), a protein expressed in response to shear stress for the purpose of enhancing vascular tone in endothelial cells (14).

Correlations between elevated CRP and coronary heart disease (CHD) have been demonstrated in cross-sectional and prospective population studies including the Women's Health Study and the Third National Health and Nutrition Examination Survey (NHANES) 1988-94 (15-17). In a prospective analysis of 28,263 women who participated in the Women's Health Study, Ridker et al. (17) found high-sensitivity (hs)-CRP was a stronger predictor of the risk of cardiovascular events than other markers associated with cardiovascular events:, the relative risk of the highest compared with the lowest quartile for this marker was 4.4 (95% confidence interval [CI]:2.2-8.9). The studies using data from the Third NHANES found that among children and young adults, CRP levels were directly correlated with body mass index (BMI) and waist-to-hip ratio (16,18).

These findings bolster evidence from other studies demonstrating IL-6 and TNF-α production by adipocytes (11,19). In an arterio-venous difference study of 39 male and female participants, concentrations of IL-6 increased with adiposity, with an estimated

15-35%, depending on time of day, of the circulating concentrations of IL-6 in healthy subjects originating from adipocytes (19). Similarly, in a cross-sectional analysis of 39 male and female subjects (11), levels of CRP were found to be significantly related to levels of IL-6 ($r=0.37$, $P<0.0005$) and TNF-α ($r=0.46$, $P<0.0001$).

**Myocytes and CRP**

C - reactive protein concentrations are largely determined by upstream production of the cytokine IL-6 (20). In a 2007 review examining the role of myokines in exercise and metabolism, Pedersen et al. (21) noted that the magnitude of increase in IL-6 during exercise is related to duration, intensity, the amount of muscle mass involved, and endurance capacity. However, investigations of this cytokine have produced mixed results about the anti- or pro-inflammatory nature of this cytokine (22). Evidence has shown that IL-1ra and IL-10, both considered anti-inflammatory cytokines, will increase in response to exercise induced IL-6 production (3). Interestingly, some studies have also suggested that IL-6 produced by myocytes, which are also often referred to as muscle cells or muscle fibers, appears to produce an anti-inflammatory response (23). This contrasts with IL-6 production by adipocytes, which appears to be associated with chronic pro-inflammatory production of IL-6 (23). Interleukin-6 produced by myocytes during exercise has also been previously shown to increase the production of IL-10 and IL-1ra and decrease the production of TNF-α and IL-1β (22,24). Thus, evidence now suggests that in response to exercise, myocytes may produce anti-inflammatory IL-6.

**Analysis of Individual Studies**

In a 2010 review by Beavers et al. (25), the effects of exercise training on chronic inflammation were summarized. In this review, several observational studies demonstrated inverse associations between self-reported PA or fitness and CRP independent of obesity. However, studies utilizing a resistance training (RT) intervention have produced mixed results. The following sections will discuss 1) studies that reported significant reductions in CRP when implementing a RT intervention (Table 1); 2) those studies that found no significant reductions when implementing a RT intervention (Table 2); and lastly 3) those examining gender and ethnicity differences in the distribution of CRP.

*Studies Reporting Lower CRP with RT*

In a 2003 study examining the relationship between inflammatory markers and exercise in 4,072 adult (≥17 years of age) participants in the Third NHANES, King et al. (33) reported inverse associations between reporting participation in various forms of exercise and having an elevated CRP level (>3 mg/L). These investigators compared the CRP levels of non- and infrequent exercisers (0-11 times/mo.) to those who engage in regular exercise (≥12 times/mo.). Analysis revealed significantly ($P<0.05$) lower likelihood of elevated CRP among those reporting participation in physical activities, which included weight lifting and calisthenics, when using non- and infrequent exercisers (0-11 times/mo.) combined as the referent group. However, following adjustments for covariates that included age, race/ethnicity, gender, BMI, smoking, and health status, this association remained significant only in those reporting jogging or aerobic dancing.

In contrast to the above-mentioned representative sample of adults, some investigators have studied disease-specific populations. For example, in 2006 White et al. (26) sought to examine whether RT in individuals with multiple sclerosis (MS) would significantly improve immune function. These investigators recruited 10 overweight (BMI=26.0 ± 7.0 kg/m$^2$) middle aged (47.0 ± 12.0 yr) female MS patients to participate in eight weeks of progressive RT (five exercises, one set, six to 15 repetitions, and two d/wk.). Pre- and post-training measurements of serum concentrations of cytokines that included CRP were taken. Following training, resting CRP levels (mg/L) were significantly reduced (before, 4.8 ± 1.7 vs. after, 3.4 ± 1.0; P=0.007) with no change in estimated body fat using a three-site skinfold measurement of subcutaneous fat (triceps, thigh, suprailium). These investigators suggested that a progressive RT program may have a significant beneficial impact on CRP concentrations in overweight women with MS.

In a study citing how little is known about the effects of RT on inflammatory biomarkers, Olson et al. (27) examined the effects of a one-year moderate intensity RT program in healthy overweight (BMI=27.0 ± 3.0 kg/m$^2$) adult (38.5 ± 5.5 yr) women. The 28 women included in this study were assigned to either a control (n=12) or RT group (n=16). Measures were taken at baseline and following one year of RT. These investigators reported no differences at baseline or following the intervention for change in body mass, BMI, fat mass or percent body fat using dual-energy x-ray absorptiometry (DXA). There was, however, a significant increase in lean body mass in the intervention group ($P<0.05$). Interestingly, no changes were demonstrated in IL-6, a known upstream determinant for increased CRP production, but significant reductions were noted for CRP

($P<0.01$) in the RT group. This study demonstrates the beneficial effects of RT on CRP levels in overweight women.

Other investigators have chosen to focus on specific ethnicities. For example, Brooks et al. (28) sought to examine whether or not RT would be an effective addition to a lifestyle intervention designed to influence variables that included CRP levels. The participants included 62 Hispanic older (66 ± 1.5 yr) obese (BMI=31.1 ± 1.1 kg/$m^2$) adults identified as community-dwelling with type 2 diabetes (T2D). Participants were randomized into a RT plus standard care group (n=31) or a standard care only control group (n=31) for 16 weeks. Measures were taken at baseline and again at 16 weeks. The RT group showed significant reductions in median CRP levels [RT: median CRP,-1.3 (interquartile range, 2.9) mg/L vs. Controls: 0.4 (2.3) mg/L, P=0.05]. Any changes in measures of adiposity went unaddressed by the study authors. In this group of older Hispanic adults with T2D, RT significantly improved CRP levels.

In contrast, the results from a study of young African American and White American male subjects produced mixed results. Heffernan et al. (29) reported that in a sample of 44 young (22.0 ± 1.0 yr) overweight (BMI=27.3 ± 1.2 kg/$m^2$) males, six weeks of RT (five to six exercises, three sets, eight to 15 rep maximum, three d/wk.) resulted in a significant ($P<0.05$) decrease in CRP levels (mg/L) in only African American subjects, a decrease that remained significant following a four week detraining period (CRP at baseline; 4.84 ± 0.9, following six weeks of training; 2.34 ± 0.5, and following four weeks of detraining; 1.91 ± 0.4). There were no significant changes in body fat for any study participants. No significant reductions in CRP were observed in the white study participants.

In a study that combined both genders, Donges et al. (30) investigated the effects of 10 weeks of exercise training on CRP in a sample of male (n=45) and female (n=57) overweight (BMI=28.7 ± 4.5 kg/$m^2$) sedentary subjects (ages not provided). Subjects were assigned to a RT group (n=35), an aerobic training (AT) group (n=41), or a control group (n=26) that remained sedentary. Interestingly, only the RT protocol resulted in a significant ($P<0.05$) attenuation of CRP levels, (CRP at baseline, 3.57 ± 2.08 mg/L, following training 2.40 ± 2.03 mg/L) a 32.8% reduction. In this study, the AT group demonstrated significant ($P<0.05$) reductions in measures of adiposity when compared to the RT group, including body mass, BMI, and DXA measured intra-abdominal fat mass. Given the resultant lack of change in adiposity in the RT group, these authors have suggested that an alteration in body composition, while beneficial, may not be necessary to reduce CRP.

In a similarly designed study, Martins et al. (31) investigated the effects of different exercise programs on CRP levels in 45 overweight (BMI=29.6 ± 4.5 kg/$m^2$) elderly (76.9 ± 7.3 yr) men (n=18) and women (n=27) randomly assigned to one of two 16 week exercise intervention groups, AT or RT, or a non-exercising control group. The training period was followed by a 16 week detraining period. Measures were taken at baseline and at the 16 and 32 week end points. These investigators reported a 10 and 51% decrease in CRP at 16 and 32 weeks for the AT group, respectively. In comparison, CRP levels decreased 11 and 39% in the RT group at the 16 and 32 week end points, respectively. The reductions in CRP were significant for both intervention groups at the 32 week end point when compared to baseline measures. Interestingly, no significant

changes were noted in the RT group for BMI. However, waist circumference (WC) significantly increased from week 16 to 32.

Using another elderly cohort, Ogawa et al. (32) investigated the impact of 12 weeks of low-intensity RT on CRP levels in 21 elderly women (85.0 ± 4.5 yr) with desirable BMI levels (BMI=21.2 ± 4.0 $kg/m^2$). C - reactive protein was among several biomarkers being measured to assess improvements in levels of inflammation at baseline and following the training period. This study did not utilize a control group. However, in comparison to baseline measures of CRP (2.44 ± 3.22), RT resulted in significantly reduced CRP levels (1.46 ± 2.22, $P<0.05$). These reductions were also reported to be significantly ($P<0.01$) associated with increased muscle thickness ($r=-0.61$). No significant changes were noted for body weight (kg), BMI, or WC. None of the results were significant after applying a Bonferroni correction. Thus, there is evidence suggesting RT interventions lead to improvements in CRP levels in women.

**Table 1. Trials Reporting Significant Reductions in CRP with RT**

| Author (Year) | N | Subjects Age (Years) | Duration | RT Intervention | RT BMI (kg/m$^2$) Before | RT BMI (kg/m$^2$) After | RT CRP (mg/L) Before | RT CRP (mg/L) After |
|---|---|---|---|---|---|---|---|---|
| White et al.(26) (2006) | 10 *Uncontrolled* | overweight women w/ MS 47.0 ± 12.0 | 8 wk.; 2 d/wk. | 1 set; 5 exercises; 6-15 reps; 50-10% MVC | 26.0 ± 7.0 | n/a | 4.8 ± 1.7 | 3.4 ± 1.0 P=0.007 |
| Olson et al.(27) (2007) | 28 (CG=12) (RT=16) | overweight women 38.5 ± 5.5 | 1 yr.; 2 d/wk. | 3 sets; 9 exercises; 8-10 reps; 8RM | 26.9 ± 3.0 | 27.5 ± 3.8 | 3.3 ± 0.4 | 3.0 ± 0.4 P<0.01 |
| Brooks et al.(28) (2007) | 62 (CG=31) (RT=31 | obese Hispanic men w/ T2D 66 ± 1.5 | 16 wk.; 3 d/wk. | 3 sets; 5 exercises; 8 reps; 60-80% 1RM | 30.9 ± 1.1 | n/a | 3.5 (9.1)[a] | 2.8 (2.8)[a] P=0.05 |
| Heffernan et al.(29) (2009) | 44 *Uncontrolled* | overweight men 22.0 ± 1.0 | 6 wk.; 3d/wk. | 3 sets; 5-6 exercises; 8-15RM | 27.3 ± 1.2 | n/a | AA, 4.84(0.9)<br>WA, 1.34(0.9) | AA, 2.34(0.5) P<0.05<br>WA, 1.70(0.5) P>0.05 |
| Donges et al.(30) (2010) | 102 (CG=26) (RT=35) (AT=41) | overweight men and women (age n/a) | 10 wk.; 3d/wk. | 2-4 sets; 5-7 exercises; 8-10 reps; 70-75% 1RM | 27.8 ± 3.9 | 28.1 ± 4.0 | 3.57 ± 2.08 | 2.40 ± 2.03 P<0.05 |
| Martins et al.(31) (2010) | 45 (CG=13) (RT=14) (AT=18) | obese men and women 76.9 ± 7.3 | 16 wk.; 3d/wk.<br>16 wk. detraining | 1-3 sets; 8 exercises; 8-15reps | 30.8 ± 5.3 | 16 wk.; 30.3 ± 5.2<br>32 wk.; 30.6 ± 5.5 | 5.61 ± 4.12 | 16 wk.; 5.02 ± 3.85<br>32 wk.; 3.40 ± 2.85 P<0.05 |
| Ogawa et al.(32) (2011) | 21 *Uncontrolled* | women 85.0 ± 4.5 | 12 wk.; ≥1d/wk. | 1-2sets; 4 exercises; 10reps | 21.2 ± 4.0 | 21.3 ± 3.8 | 2.44 ± 3.22 | 1.46 ± 2.22 P<0.05 |

Abbreviations: kg=kilograms; m=meters; hr.=hours; d=day; wk.=week; mo.=month; CRP=C - reactive protein; CG=control group; RT=resistance training; AT=aerobic training; MVC=maximal voluntary contraction; RM= repetition maximum; BMI=body mass index; yr = years; T2D=Type 2 Diabetes; MS= Multiple Sclerosis; AA=African American; WA=White American
values expressed as mean ± SD or mean (SE)
[a] median (Interquartile Range)

*Studies Reporting No Change in CRP with RT*

A 2006 study by Klimcakova et al. (34) examined whether RT in obese (BMI=33.6 ± 1.2 kg/m$^2$) adult (50.4 ± 2.3 yr) males (n=12) is associated with changes in levels of adipokines that may be involved in development of insulin resistance. Blood samples were taken at baseline and following three months of RT. Body weight remained

unchanged during training. Study results revealed no significant changes in plasma levels of adipokines or CRP. In this small group of obese male subjects, RT resulted in improved insulin sensitivity; however, this change was not associated with any corresponding reduction in CRP.

Kohut et al. (35) hypothesized that a long-term exercise intervention among older male and female adults would reduce inflammatory cytokines such as CRP, and that this potential reduction would be mediated, in part, by improvements in psychosocial factors and possibly adrenergic receptor mechanisms. Adults (≥64 yr) were randomized into either an AT group or a flexibility/strength exercise treatment group. The protocol required participation three d/wk, 45 min/day, for 10 months. These investigators reported significant reductions in serum CRP, IL-6, and IL-18 in those assigned to the AT group when compared to the flexibility/strength treatment group.

Overtraining protocols using younger male subjects have also failed to reveal significant reductions in CRP. Peake et al. (36) recruited 10 healthy young men who reported not being involved in a RT program and compared the changes in markers of muscle damage and systemic inflammation following submaximal and maximal lengthening muscle contractions of elbow flexors. The submaximal training required 10 sets of 60 lengthening contractions at 10% maximum isometric strength with one-minute of rest between sets. The maximal training required 10 sets of three lengthening contractions at 100% maximum isometric strength with three minutes of rest between sets. The contractions were performed using an isokinetic dynamometer. The trials were separated by a minimum of two weeks and opposite arms were used for each trial. Venous blood samples were drawn before the trial, immediately after, at one hour and

three hours following completion, and one to four days following each trial. Following both trials, CRP remained unchanged. Interestingly, it was reported that there were no differences in markers of inflammation when comparing the trials, even though greater muscle damage was reported following maximal versus submaximal contractions.

In a trial designed to evaluate progressive RT, Misra et al. (37) recruited 30 (22 male, 8 female) Asian Indian adults (40.8 ± 8.1yr) with T2D. These subjects participated in a 12 week RT program focused on six muscle groups (biceps flexion, shoulder flexion, finger grip, hip flexion, knee extension, and heel raise) implementing two set, 10 repetition protocols. No significant changes were noted in CRP levels. However, this moderate-intensity three month RT protocol did result in significant improvement in markers that included insulin sensitivity, glycemia, and lipids.

In other highly disease-specific populations, AT and RT interventions have revealed mixed results on CRP levels. Utilizing biomarkers in subjects infected with Human Immunodeficiency Virus, Lindegaard et al. (38) evaluated the effects of RT and AT on insulin sensitivity and fat distribution. Subjects for this study included 20 HIV-infected men, who were randomly assigned to RT (n=10) or AT (n=8, 2 withdrew) three times/wk for 16 weeks. The RT consisted of eight exercises (leg curl, pull-down, seated leg press, chest press, seated rows, leg extension, abdominal crunch, and back extension) using LifeFitness RT machines for 45–60 minutes. Results revealed significant ($P<0.05$) reductions in CRP concentrations only for those assigned to the AT group.

In a study noting that inflammatory markers like CRP, hepatic enzymes, and physical inactivity are often associated with the metabolic syndrome (39), Levinger et al. (40) examined whether these markers are correlated with risk factors for metabolic

syndrome by studying the effects of RT in individuals with a high number of metabolic risk factors (2.9 ± 0.8) and those with a low number (0.5 ± 0.5). This study included 28 male and 27 female adults (50.8 ± 6.5 yr). Blood samples were obtained at baseline and following 10 weeks of RT. The study protocol included three sets of seven exercises (chest press, leg press, lateral pull-down, triceps push-down, knee extension, seated row and biceps curl) three d/wk.. Intensity was gradually increased from 40-50% to 75-85% of one repetition maximum (1RM=largest load that an individual can lift or move in a single effort). At baseline, those with a high number of metabolic risk factors had significantly ($P<0.05$) higher levels of IL-6 (33.9%) and CRP (57.1%) when compared with those with a low number of risk factors. Resistance training did not significantly change any of the inflammatory markers being studied. Though a high number of metabolic risk factors were associated with significantly increased levels of CRP, RT failed to reduce these levels.

In a study designed to examine different types of training and inflammatory markers, Libardi at al. (41) evaluated the effects of 16 weeks of RT, AT, or concurrent training (CT) on CRP levels and functional capacity in sedentary middle-aged men. Following randomization, subjects performed three weekly sessions lasting approximately one hour for 16 weeks. Maximal strength was tested using the bench press and leg press. Though maximal strength was increased following the 16 week training period, there were no significant differences in CRP when comparing measures collected at baseline to those at the study conclusion for any of the three assignment groups.

Swift et al. (42) also noted that though AT has been shown to improve CRP, there remains limited data evaluating other training modalities, such as RT or training that

combines RT and AT. Participants recruited for the purpose of investigating these different types of training included 204 older male and female adults with T2D (42). Subjects were randomized to an AT, RT, or a CT group for nine months. Participants in the RT group exercised three d/wk and completed two sets, 10 to 12 reps, of four upper body exercises (bench press, seated row, shoulder press, and lat pull down), three sets of three leg exercises (leg press, extension, and flexion), and two sets of abdominal crunches and back extensions. Concentrations of CRP were evaluated at baseline and at follow-up. Similar to the previously described study by Libardi et al. (41), CRP was not significantly reduced for any of the training modalities when compared to controls.

**Table 2. Trials Reporting No Significant Reductions in CRP with RT**

| Author (Year) | N | Subjects Age (Years) | Duration | RT Intervention | RT BMI ($kg/m^2$) Before | RT BMI ($kg/m^2$) After | RT CRP (mg/L) Before | RT CRP (mg/L) After |
|---|---|---|---|---|---|---|---|---|
| Klimcakova et al.(34) (2006) | 12 *Uncontrolled* | obese men 50.4 ± 2.3 | 12 wk.; 3d/wk. | 1 set; 17 exercises; 12-15 reps; 60-70% 1RM | 33.6 ± 3.9 | 33.7 ± 4.0 | 3.3 ± 2.2 | 2.9 ± 1.7 |
| Kohut et al.(35) (2006) | 87 RT=47 AT=40 | overweight men and women 70.1 ± 5.1 | 10 mo.; 3d/wk. | 10-15 reps; self-directed using various machines | 29.3 ± 0.8 | 29.0 ± 0.8 | > 4.0 | ≈ 3.5 to 4.5 |
| Peake et al.(36) (2006) | 10 *Crossover Design* | men 22.9 ± 4.7 | 4d. | *Submax*; 10 sets; 60 reps; 10 % MIS *Max*; 10 sets; 3 reps; 100% MIS | n/a | n/a | *Submax*; 0.2 ± 0.3 *Max*; 0.5 ± 0.7 | *Submax*; 0.5 ± 1.3 *Max*; 0.5 ± 0.8 |
| Misra et al.(37) (2008) | 30 *Uncontrolled* | men and women w/ T2D 40.8 ± 8.1 | 12wk.; 3d/wk. | 2 sets; 6 exercises; 10 reps; | 24.1 ± 3.9 | 24.1 ± 3.7 | n/a | n/a |
| Lindegaard et al.(38) (2008) | 33 CG=15 RT=10 AT=8 | men w/ HIV (n=18) Healthy CG (n=15) (age n/a) | 16 wk.; 3d/wk. | 3-4 sets; 8 exercises; 8-12 reps; 50-80% 1RM | 23.4 ± 2.5 | n/a | 1.54 (1.0-2.37)[a] | 0.11 (0.18-0.35)[c] |
| Levinger et al.(40) (2009) | 55 HiMFT=15 HiMFC=15 LoMFT=12 LoMFC=13 | men and women 50.8 ± 6.5 | 10 wk.; 3d/wk. | 3 sets; 7 exercises; 8-20 reps; 40-85% 1RM | n/a | n/a | HiMF 2.8 ± 1.7 LoMF 1.2 ± 1.0 | HiMFT 0.3 ± 2.5[b] HiMFC 1.2 ± 3.7[b] LoMFT 0.4 ± 0.9[b] LoMFC -0.2 ± 0.7[b] |
| Libardi et al.(41) (2011) | 47 RT=11 AT=12 CT=11 CG=13 | overweight men 49.0 ± 5.3 | 16 wk.; 3d/wk. | 3 sets; 8 exercises; 8-10 reps; 8-10RM | 27.1 ± 4.0 | 27.3 ± 3.7 | 1.7 ± 1.3 | 2.7 ± 1.9 |
| Swift et al.(42) (2011) | 204 CG=37 AT=50 RT=58 CT=59 | obese men and women 57.3 ± 8.1 | 36 wk.; 3d/wk. | 2-3sets; 9 exercises; 10-12 reps; | 34.1 ± 5.4 | n/a | 4.1 ± 4.5 | -0.03 (1.08-1.02)[c] |

Abbreviations: kg=kilograms; m=meters; s.=seconds; hr.=hours; d=day; wk.=week; mo.=month; CRP= C - reactive protein; CG=control group; RT=resistance training; AT=aerobic training; ES=electrical stimulation; MVC=maximal voluntary contraction; RM= repetition maximum; BMI=body mass index; yr= years; T2D=Type 2 Diabetes; MS=Multiple Sclerosis; AA=African American; WA=White American; HiMF=high number of metabolic risk factors (2.9 ± 0.8); LoMF=low number of metabolic risk factors (0.5 ± 0.5); MIS=Maximum Isometric Strength; CI=confidence interval; SCHF=Stable Chronic Heart Failure unless otherwise noted, values expressed as mean ± SD or mean (SE)

[a] mean (range)

[b] mean change ± SD

[c] mean change (95% CI)

*Gender Differences in CRP and PA*

Several studies have revealed differences in CRP levels when comparing males and females or simply chose sex-specific CRP cut-points because women inherently have increased CRP in comparison to men. For example, in a 2002 study using data from the Third NHANES (1988-1994) the association between PA and CRP was investigated by Ford (43) using a sample that included 13,748 male and female adults (≥20 yr) using sex-specific CRP cut-points (≥85th percentile). Following adjustment for age, sex, ethnicity, education, work status, smoking status, cotinine concentration, hypertension, BMI, waist-to-hip (WTH) ratio, high-density lipoprotein cholesterol (HDL-C), and aspirin use, the odds ratio (OR) for elevated CRP concentration were 0.98 (95% CI 0.78-1.23), 0.85 (95% CI 0.70-1.02), and 0.53 (95% CI 0.40-0.71) for participants who reported engaging in light, moderate, and vigorous PA respectively during the previous month when compared to a referent group reporting no LTPA.

When stratified by gender, several other studies analyzing the distribution of elevated CRP levels (>3 mg/L) or the relationship between PA, CRP, and adiposity have produced mixed results (44-49). In a study by Lear et al. (50) the effects of ethnicity and gender have on the relationship between CRP, BMI, WC, and WTH was investigated in women (n=47) and men (n=44) of Chinese decent and women (n=52) and men (n=39) of European descent recruited from a local hospital staff. Results revealed significantly lower CRP in Chinese men and women when compared with Europeans; however, this difference was no longer present after correction for either BMI or WC. In female participants, BMI ($r=0.55$, $P<0.01$) and WC ($r=0.59$, $P<0.01$) were significantly correlated with CRP. Gender also significantly interacted with WC to predict CRP following adjustment for age, smoking status, alcohol, and BMI ($P<0.05$). Interestingly,

the interaction between gender and BMI as a predictor of CRP was not significant in this population. Moreover, these investigators reported that once WC exceeded 70 cm, CRP levels increased at a greater rate in women than in men.

Studies that investigated the frequency of participation in PA have also produced mixed results when stratified by gender. In 2004, Albert et al. (45) reported that in male (n=1,732) and female (n=1,101) adult (60.8 ± 12yr for both men and women) enrollees in the Pravastatin Inflammation/CRP Evaluation (PRINCE) study, concentrations of CRP were significantly lower among men who reported a high level of strenuous aerobic PA (≥4 times/wk) when compared with men who reported rarely performing strenuous aerobic PA (rarely/<once/wk). Furthermore, this association revealed in men remained significant following adjustment for age, HDL-C, smoking status, BMI, diabetes, aspirin use, and systolic blood pressure (SBP) (P for trend<0.001). However, a significant relationship was not found in women (P for trend=0.38).

Interestingly, data collected from adult (65.3 ± 9.0 yr) male (n=1,926) and female (n=2,260) participants in the English Longitudinal Study of Ageing (ELSA) have also demonstrated independent associations between CRP and muscle strength that appear to be stronger in women (51). In these participants, strength was assessed using hand grip strength and lower body strength (time required to complete five chair stands). Results revealed that about 33% of the study participants had an elevated CRP level (≥3 mg/L). Following adjustment for age, smoking, PA, education, and inflammatory diseases, elevated CRP levels were associated with poorer hand grip strength and lower body strength in women but only with lower body strength in men.

In a more recent 2011 study using data collected in older (>60 yr) male (n=422) and female (n=445) adult participants in the NHANES 2001-2002, Canon et al. (46) reported that elevated levels of CRP may mediate the relationship between cognitive function and muscle quality in females but not males. The stated purpose of this study was to examine the sex-specific differences in the relationship between low muscle quality, impaired cognitive functioning, and the possible role of inflammatory markers. Muscle quality was calculated as isokinetic strength per unit muscle mass and skeletal muscle mass of the legs was measured using DXA. Interestingly, CRP was found to have a significant negative association with cognitive functioning for females but not males. These researchers concluded that the associations between sarcopenia and lower cognitive functioning may be partly due to systemic inflammation.

In a study seeking to determine the race and gender differences in the distribution of CRP levels, Khera et al. (48) measured the CRP levels in 2,749 white and African American middle-aged (30-65 yr) enrollees in the Dallas Heart Study. Results of this study revealed significantly higher CRP levels in African American subjects when compared to white subjects (median, 3.0 vs. 2.3 mg/L; $P<0.001$). Analysis also revealed higher CRP levels in women when compared to men (median, 3.3 vs. 1.8 mg/L; $P<0.001$). The percentage of subjects in this population that would be considered high risk according to CRP level (>3 mg/L) was 31%, 40%, 51%, and 58% in white men, black men, white women, and black women, respectively. Following adjustment for CV risk factors, hormone replacement therapy, statin use, and BMI, when compared to a referent group of white men, an elevated CRP level (>3 mg/L) was more common in white women (OR 1.6; 95% CI 1.1-2.5) and black women (OR 1.7; 95% CI 1.2-2.6) but

not in black men. Thus, significant race and gender differences exist in this population when examining the distribution of CRP.

Other investigators have also suggested that the increases in inflammatory biomarkers associated with obesity might be modified by gender. Thorand et al. (52) sought to investigate these relationships in men (n=641) and women (n=597) aged 55-74 years who participated in the Kooperative Gesundheitsforschung in der Region Augsburg (KORA) survey 2000. This study was conducted in the area of Augsburg, Germany. In this population, all measures of adiposity (fat mass percentage, BMI, WC, WTH) were reported to be highly correlated with CRP in both male and female study participants. However, analysis revealed a higher percentage of variability in CRP concentrations was explained by body composition in women when compared to men. Furthermore, the relevance of individual measures varied by sex. For example, fat mass percentage explained the highest percentage of variability of CRP in women, 18.2%, and WTH explained the highest percentage of the variability of CRP in men, 6.2%. Interestingly, WC explained the highest percentage of variability in IL-6, a known upstream determinant of increased hepatic production of CRP, in women, 3.7%, and men, 1.8%, ($P<0.001$ for both). Thus, while adiposity was strongly associated with CRP levels in men and women, the association was reported to be considerably stronger in women.

Gender differences have also been reported when investigating the relationships among adiposity, inflammation, PA, fitness, and fatigue (53). In 127 overweight (BMI=28.0 $kg/m^2$) community-dwelling older (70 yr) adults, a significant ($P<0.05$) relationship between CRP ($r=0.29$) and fatigue has been reported by Valentine et al. (53) in women, but not men. In contrast, CRP levels of female study participants were 40%

higher and adiposity 12% higher in those reporting fatigue when compared to those reporting no fatigue. A similar relationship was not revealed in male study participants.

When investigating the relationship between fitness and CRP, some evidence has also suggested the existence of gender differences in children and young adult populations. In a study published in 2003, Isasi et al. (44) investigated the association between treadmill assessed fitness levels and CRP in children and young adult males (n=95) and females (n=110) aged six to 24 years who participated in the Columbia University Biomarkers Study (1994-1998). Following adjustment for age, ethnicity, BMI, and family history of ischemic heart disease, fitness remained inversely associated with CRP levels only in males ($\beta$=-0.02; SE= 0.01, P=0.03).

**Summary**

Evidence suggests that PA may reduce inflammation, which is a critical process in the pathogenesis of cardiovascular disease. However, the association between MSA and CRP is less clear. Since CRP has shown efficacy in predicting incidence of CVD (4), examining the possible mediating associations between MSA and CRP may be particularly important for men and women who do not currently perform any volume of MSA, or for those performing volumes of MSA that do not meet the DHHS recommendation (MSA $\geq$2 d/wk).

The reviewed studies included a variety of populations. Characteristics of study participants varied according to gender, age range, BMI class, and the presence of known inflammatory conditions. Consequently, it is very difficult to generalize the results from these studies to the broader population. Thus, it is important to examine the associations

between CRP and MSA using population-based or nationally representative data. The present study may also add to the current literature by demonstrating a more pronounced association among females and ethnic subgroups (47,48), as well as those reporting adiposity as a strong mediating factor (16,49,52). Moreover, since MSA participation is estimated to be lower in female subgroups (54,55), revealing a mediating association may become increasingly important for allied health care professionals who are able to promote MSA participation within these groups.

**References**


1. Jialal I, Devaraj S, Venugopal SK. C-reactive protein: risk marker or mediator in atherothrombosis? Hypertension 2004;44:6-11.
2. Kaptoge S, Di Angelantonio E, Lowe G, et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet 2010;375:132-40.
3. Petersen AM, Pedersen BK. The anti-inflammatory effect of exercise. J Appl Physiol 1985;98:1154-62.
4. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation 2003;107:363-9.
5. Tillett WS, Francis T. Serological Reactions in Pneumonia with a Non-Protein Somatic Fraction of Pneumococcus. J Exp Med 1930;52:561-71.
6. Black S, Kushner I, Samols D. C-reactive Protein. J Biol Chem 2004;279:48487-90.
7. Ablij H, Meinders A. C-reactive protein: history and revival. Eur J Intern Med 2002;13:412.
8. Yeh ET, Willerson JT. Coming of age of C-reactive protein: using inflammation markers in cardiology. Circulation 2003;107:370-1.
9. Thompson D, Pepys MB, Wood SP. The physiological structure of human C-reactive protein and its complex with phosphocholine. Structure 1999;7:169-77.
10. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. J Clin Invest 2003;111:1805-12.
11. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial

dysfunction: a potential role for cytokines originating from adipose tissue? Arterioscler Thromb Vasc Biol 1999;19:972-8.

12. Floyd-Smith G, Whitehead AS, Colten HR, Francke U. The human C-reactive protein gene (CRP) and serum amyloid P component gene (APCS) are located on the proximal long arm of chromosome 1. Immunogenetics 1986;24:171-176.
13. Zwaka TP, Hombach V, Torzewski J. C-reactive protein-mediated low density lipoprotein uptake by macrophages: implications for atherosclerosis. Circulation 2001;103:1194-7.
14. Hambrecht R, Adams V, Erbs S, et al. Regular physical activity improves endothelial function in patients with coronary artery disease by increasing phosphorylation of endothelial nitric oxide synthase. Circulation 2003;107:3152-8.
15. Ridker PM, Buring JE, Shih J, Matias M, Hennekens CH. Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. Circulation 1998;98:731-3.
16. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-reactive protein levels in overweight and obese adults. JAMA 1999;282:2131-5.
17. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000;342:836-43.
18. Ford ES, Galuska DA, Gillespie C, Will JC, Giles WH, Dietz WH. C-reactive protein and body mass index in children: findings from the Third National Health and Nutrition Examination Survey, 1988-1994. J Pediatr 2001;138:486-92.

19. Mohamed-Ali V, Goodrick S, Bulmer K, Holly JM, Yudkin JS, Coppack SW. Production of soluble tumor necrosis factor receptors by human subcutaneous adipose tissue in vivo. Am J Physiol 1999;277:E971-5.

20. Castell JV, GÃ³mez-LechÃ³n MJ, David M, et al. Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. FEBS letters 1989;242:237-239.

21. Pedersen BK, Åkerström, TC, Nielsen AR, Fischer CP. Role of myokines in exercise and metabolism. Journal of applied physiology 2007;103:1093-1098.

22. Mathur N, Pedersen BK. Exercise as a mean to control low-grade systemic inflammation. Mediators of inflammation 2009;2008:

23. Pedersen BK, Febbraio M. Muscle-derived interleukin-6--a possible link between skeletal muscle, adipose tissue, liver, and brain. Brain Behav Immun 2005;19:371-6.

24. Brandt C, Pedersen BK. The role of exercise-induced myokines in muscle homeostasis and the defense against chronic diseases. BioMed Research International 2010;

25. Beavers KM, Brinkley TE, Nicklas BJ. Effect of exercise training on chronic inflammation. Clin Chim Acta 2010;411:785-93.

26. White LJ, Castellano V, Mc Coy SC. Cytokine responses to resistance training in people with multiple sclerosis. J Sports Sci 2006;24:911-4.

27. Olson TP, Dengel DR, Leon AS, Schmitz KH. Changes in inflammatory biomarkers following one-year of moderate resistance training in overweight women. Int J Obes (Lond) 2007;31:996-1003.

28. Brooks N, Layne JE, Gordon PL, Roubenoff R, Nelson ME, Castaneda-Sceppa C. Strength training improves muscle quality and insulin sensitivity in Hispanic older adults with type 2 diabetes. Int J Med Sci 2007;4:19-27.

29. Heffernan KS, Jae SY, Vieira VJ, et al. C-reactive protein and cardiac vagal activity following resistance exercise training in young African-American and white men. Am J Physiol Regul Integr Comp Physiol 2009;296:R1098-105.

30. Donges CE, Duffield R, Drinkwater EJ. Effects of resistance or aerobic exercise training on interleukin-6, C-reactive protein, and body composition. Med Sci Sports Exerc 2010;42:304-13.

31. Martins RA, Neves AP, Coelho-Silva MJ, Verissimo MT, Teixeira AM. The effect of aerobic versus strength-based training on high-sensitivity C-reactive protein in older adults. Eur J Appl Physiol 2010;110:161-9.

32. Ogawa K, Sanada K, Machida S, Okutsu M, Suzuki K. Resistance exercise training-induced muscle hypertrophy was associated with reduction of inflammatory markers in elderly women. Mediators Inflamm 2011;2010:171023.

33. King DE, Carek P, Mainous AG, 3rd, Pearson WS. Inflammatory markers and exercise: differences related to exercise type. Med Sci Sports Exerc 2003;35:575-81.

34. Klimcakova E, Polak J, Moro C, et al. Dynamic strength training improves insulin sensitivity without altering plasma levels and gene expression of adipokines in subcutaneous adipose tissue in obese men. J Clin Endocrinol Metab 2006;91:5107-12.

35. Kohut ML, McCann DA, Russell DW, et al. Aerobic exercise, but not flexibility/resistance exercise, reduces serum IL-18, CRP, and IL-6 independent of Î²-blockers, BMI, and psychosocial factors in older adults. Brain, Behavior, and Immunity 2006;20:201-209.

36. Peake JM, Nosaka K, Muthalib M, Suzuki K. Systemic inflammatory responses to maximal versus submaximal lengthening contractions of the elbow flexors. Exerc Immunol Rev 2006;12:72-85.

37. Misra A, Alappan NK, Vikram NK, et al. Effect of supervised progressive resistance-exercise training protocol on insulin sensitivity, glycemia, lipids, and body composition in Asian Indians with type 2 diabetes. Diabetes Care 2008;31:1282-7.

38. Lindegaard B, Hansen T, Hvid T, et al. The effect of strength and endurance training on insulin sensitivity and fat distribution in human immunodeficiency virus-infected patients with lipodystrophy. J Clin Endocrinol Metab 2008;93:3860-9.

39. Churilla JR, Fitzhugh EC, Thompson DL. The Metabolic Syndrome: How Definition Impacts the Prevalence and Risk in U.S. Adults: 1999-2004 NHANES. Metab Syndr Relat Disord 2007;5:331-42.

40. Levinger I, Goodman C, Peake J, et al. Inflammation, hepatic enzymes and resistance training in individuals with metabolic risk factors. Diabet Med 2009;26:220-7.

41. Libardi CA, De Souza GV, Cavaglieri CR, Madruga VA, Chacon-Mikahil MP. Effect of resistance, endurance, and concurrent training on TNF-alpha, IL-6, and CRP. Med Sci Sports Exerc 2011;44:50-6.

42. Swift DL, Johannsen NM, Earnest CP, Blair SN, Church TS. Effect of exercise training modality on C-reactive protein in type 2 diabetes. Med Sci Sports Exerc 2011;44:1028-34.

43. Ford ES. Does exercise reduce inflammation? Physical activity and C-reactive protein among U.S. adults. Epidemiology 2002;13:561-8.

44. Isasi CR, Deckelbaum RJ, Tracy RP, Starc TJ, Berglund L, Shea S. Physical fitness and C-reactive protein level in children and young adults: the Columbia University BioMarkers Study. Pediatrics 2003;111:332-8.

45. Albert MA, Glynn RJ, Ridker PM. Effect of physical activity on serum C-reactive protein. Am J Cardiol 2004;93:221-5.

46. Canon ME, Crimmins EM. Sex differences in the association between muscle quality, inflammatory markers, and cognitive decline. J Nutr Health Aging 2011;15:695-8.

47. Majka DS, Chang RW, Vu TH, et al. Physical activity and high-sensitivity C-reactive protein: the multi-ethnic study of atherosclerosis. Am J Prev Med 2009;36:56-62.

48. Khera A, McGuire DK, Murphy SA, et al. Race and gender differences in C-reactive protein levels. J Am Coll Cardiol 2005;46:464-9.

49. Geffken DF, Cushman M, Burke GL, Polak JF, Sakkinen PA, Tracy RP. Association between physical activity and markers of inflammation in a healthy elderly population. Am J Epidemiol 2001;153:242-50.

50. Lear SA, Chen MM, Birmingham CL, Frohlich JJ. The relationship between simple anthropometric indices and C-reactive protein: ethnic and gender differences. Metabolism 2003;52:1542-6.

51. Hamer M, Molloy GJ. Association of C-reactive protein and muscle strength in the English Longitudinal Study of Ageing. Age (Dordr) 2009;31:171-7.

52. Thorand B, Baumert J, Doring A, et al. Sex differences in the relation of body composition to markers of inflammation. Atherosclerosis 2006;184:216-24.

53. Valentine RJ, McAuley E, Vieira VJ, et al. Sex differences in the relationship between obesity, C-reactive protein, physical activity, depression, sleep quality and fatigue in older adults. Brain Behav Immun 2009;23:643-8.

54. Carlson SA, Fulton JE, Schoenborn CA, Loustalot F. Trend and prevalence estimates based on the 2008 Physical Activity Guidelines for Americans. Am J Prev Med 2010;39:305-13.

55. Churilla JR, Magyari PM, Ford ES, Fitzhugh EC, Johnson TM. Muscular strengthening activity patterns and metabolic health risk among US adults. J Diabetes 2011;4:77-84.

# Chapter Three: Methodology

The purpose of this study was to examine the associations between self-reported volumes of muscle strengthening activities (MSA) and elevated C – reactive protein (CRP) in adult participants of the National Health and Nutrition Examination Survey (NHANES) when stratified by gender. Emphasis in this study was placed on determining whether volumes of MSA meeting the 2008 Department of Health and Human Services (DHHS) recommendation (1) was associated with significantly lower odds of elevated CRP and the potential mediating effects of waist circumference on this association. This section provides the details of the methodology that was utilized to answer the research questions in this study.

**Data Collection**

This study utilized six years of data from the 1999-2004 NHANES. The NHANES is a continuous survey conducted by the National Center for Health Statistics (2). The NHANES was designed to provide national estimates of the health and nutritional status of non-institutionalized United States (U.S.) civilians over the age of two months. The NHANES also over-samples persons 60 and older, African Americans, and Hispanics to produce more reliable statistics. The participant interviews take place in the home of the survey participant. Participants who provided anthropometric measurements and biomarkers attended an examination session in one of the pre-equipped mobile examination centers (MEC). The study teams that operate these MEC units and collect these various measurements include a physician, medical technicians, and interviewers.

**Sampling Design**

The NHANES data were obtained using a complex, multistage probability sampling design. The sample selection for the NHANES follows four stages. First is the selection of primary sampling units (PSUs). These PSUs are usually counties or small groups of adjacent counties. Second is the selection of segments within these PSUs. These are made up of the equivalent of a city block or a group of blocks that contain a group of households. Third, households within these segments are randomly selected. Lastly, individuals within these households are selected at random to participate in the household interview section of the NHANES and possibly attend the MEC unit for the provision of other measurements and the serum necessary to examine biomarkers.

The design and weighting methods utilized in NHANES have been consistent over the history of the survey. The sampling weights are used to account for 1) the probability of selection; 2) the oversampling of African Americans, Mexican Americans, persons with low income, adolescents aged 12-19 years, persons aged ≥60 years; and 3) non-response rates. Thus, the weights utilized in the analyses in this study were used to account for the complex survey design, which including oversampling, survey nonresponse, and post-stratification to match the population control totals for each sampling subdomain. This last adjustment makes the weighted counts the same as an independent count of the U.S. 2000 Census. This step was important to ensure that the resultant estimates are representative of the U.S. population. A six year weight was created for the sub-sample of survey participants who attended the MEC. Creating the necessary six-year weight (WTSMEC6YR) was done while merging the six years of

survey data collected from 1999-2004 using SAS 9.2 (3). The SAS coding used to create the sample weight was as follows:

if sddsrvyr in (1,2) then MEC6YR = 2/3 *WTMEC4YR; ***1999-2002***;

If sddsrvyr=3 then MEC6YR = 1/3 *WTMEC2YR; ***2003-2004***;

Note, whenever utilizing the survey cycle variable (SDDSRVYR) 1=1999-2000, 2=2001-2002, and 3=2003-2004.

**Subjects**

The total 1999-2004 NHANES sample size was 31,126, ages two months and above. For this study, the final sample consisted of 9,135 U.S. adults (≥20 years of age). The sample for this study met the following conditions: 1) adult men and women ≥20 years of age; 2) had provided a serum blood sample in a MEC; 3) if female, not pregnant; and 4) had provided complete data on all other variables of interest.

**Study Measures**

Dependent measure(s): C - reactive protein

The dependent variable in this study was an elevated CRP concentration (3 mg/L<CRP≤10 mg/L) as defined by the Centers for Disease Control and Prevention and the American Heart Association (CDC/AHA) (4). C - reactive protein concentrations were measured using latex-enhanced nephelometry (N high-sensitivity CRP assay) on a Dade Behring II nephelometer at the University of Washington Medical Center. Seattle, Washington (5).

Primary Independent Measure(s): Muscle Strengthening Activity

The primary independent variable in this study was calculated from 'self-reported' MSA patterns. The final sample provided responses to the following items from the physical activity questionnaire file item PAD440: *Over the past 30 days, did {you/SP} do any physical activities specifically designed to strengthen {your/his/her} muscles such as lifting weights, push-ups or sit-ups? Include all such activities even if you have mentioned them before in the past 12 months*. The sample also provided responses to physical activity questionnaire file item PAD460: *Over the past 30 days, how often did you do these activities? [Activities designed to strengthen {your/his/her} muscles such as lifting weights, push-ups or sit-ups.]* The MSA variable was created with three categories: no MSA, some MSA, and meeting the DHHS recommendations. No MSA was coded as 0 d/wk, some MSA as ≥1 to <2 d/wk, and meeting the recommendation as ≥2 d/wk.

**Other Independent Measures**

The potential confounding variables that were controlled for in this study included the following:

*Age*

Age was divided into three categories: 20–39, 40–59, and ≥60 years.

*Race/Ethnicity*

Participants were classified as one of four race/ethnicity groups. The four categories were: non-Hispanic white, non-Hispanic black, Mexican American, and other.

*Education*

Education was categorized into three levels: less than high school, high school graduate, and more than high school.

*Serum Cotinine*

Current evidence using data from the 1999-2004 NHANES has suggested that the use of the conventional cut-point of 14 ng/mL overestimates the number of nonsmokers in the U.S. adult population (6). Thus, the newly recommended lowered cut-point of 3 ng/mL was used to create a dichotomous variable of ≥3 ng/mL (yes/no) to distinguish smokers from nonsmokers.

*History of Cardiovascular Disease*

A dichotomous history of cardiovascular disease variable was created based on survey participant responses (yes/no) to a series of questions located within the medical conditions questionnaire (MCQ) portion of the NHANES. These questions included the following:

> MCQ160B: *"Has a doctor or health professional ever told {you/SP} that {you/s/he} ...had congestive heart failure?"*
>
> MCQ160C: *"Has a doctor or health professional ever told {you/SP} that {you/s/he} ...had coronary heart disease?"*
>
> MCQ160D: *"Has a doctor or health professional ever told {you/SP} that {you/s/he} ...had angina, also called angina pectoris?"*

MCQ160E: *"Has a doctor or health professional ever told {you/SP} that {you/s/he} ...had a heart attack (also called myocardial infarction)?"*

MCQ160F: *"Has a doctor or health professional ever told {you/SP} that {you/s/he} ...had a stroke?"*

*Hypertension*

A dichotomous hypertension variable was created based on the National Cholesterol Education Panel Adult Treatment Panel III (NCEP ATP III) criteria (7): systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg (yes/no) or current use of blood pressure lowering medications.

*High-density Lipoprotein Cholesterol*

A dichotomous high-density lipoprotein cholesterol (HDL-C) variable was created based on the NCEP ATP III criteria (7): <40 mg/dL in men (yes/no) or <50 mg/dL in women (yes/no).

*Non-High-density Lipoprotein Cholesterol*

The use of non-HDL-C as a covariate was considered advantageous since it only requires the measurement of total cholesterol (TC) and HDL-C. The formula is as follows: Non-HDL-C=TC-HDL-C. These variables do not require a fasting sample. In contrast, the measurement of LDL-C was only available in the fasting subsample (8). Non-HDL-C categories were created based on NCEP ATP III defined goals (7). These four non-HDL-C (mg/dL) categories included levels <130, 130-159, 160-189, and >189 mg/dL.

*Arthritis*

Arthritis status was dichotomized (yes/no) based on participants' survey response to the following question located within the MCQ file:

> MCQ160A: *"Has a doctor or other health professional ever told you that {you/SP} that {you/s/he} had arthritis?"*

*Aspirin*

The use of aspirin has been previously described as a potential agent for lowering CRP levels (9). For this analysis, reporting the use of aspirin was dichotomized (yes/no) based on participant responses to the following question located in the analgesic medications (pain relievers) questionnaire file:

> RXQ300: *"{Have you/ has SP} ever taken any of these prescription or over-the counter pain relievers nearly every day for the last month?"*
>
> RXQ310: Code 10 - Aspirin - also buffered aspirin products such as Anacin, Bayer, Bufferin, Midol, Ascripton, Ecotrin, Pabrin and Alka Seltzer

*Glycohemoglobin*

Three glycohemoglobin (HbA1c %) diabetes status categories were created based on the American Diabetes Association definition (10). These categories included normal (<5.7%), pre-diabetes (5.7% to 6.4%), and those with diabetes (≥6.5%).

*Hormone Replacement Therapy*

In gender stratified analyses, use of a dichotomous (yes/no) female hormone therapy variable was used as an additional covariate in analyses utilizing female participants. This variable was based on responses to the following item from the reproductive health questionnaire file: RHQ540: *"{Have you/Has SP} ever used female hormones such as estrogen and progesterone? Please include any forms of female hormones, such as pills, cream, patch, and injectables, but do not include birth control methods or use for fertility."*

*Waist Circumference*

Waist circumference (WC) was examined continuously in an effort to account for all waist circumference measurements and thus varying degrees of central adiposity. Waist circumference was measured using steel tape just above the uppermost lateral border of the right ilium to the nearest millimeter.

**Data Analysis**

The data in this study were initially managed using SAS 9.2 (3). SAS was used to conduct both complex variable recodes and data coding validation. SAS-callable SUDAAN (11) was then used to conduct the analysis, incorporating sampling weights within the context of the correlated multi-stage complex sampling design inherent to NHANES. Participants who responded 'don't know/not sure', refused to answer, or had missing responses for any of the questions or measures were excluded from the analyses. Logistic regression models were stratified by gender and adjusted for age, race,

education, serum cotinine, HDL-C, non-HDL-C, arthritis, aspirin use, hypertension, history of CVD, HbA1c %, hormone therapy in females, and WC. Best fit models were created using a forward selection method based on the presence or absence of significant Wald f-test results. The resultant odds ratios were used to examine the associations between CRP and each of the remaining independent variables.

**Limitations**

The present study has several inherent limitations due to the design. Consequently, all findings must be interpreted with caution. These limitations include the use of the NHANES MSA data in these analyses which were collected from 1999-2004. Thus, the analyzed data may not be reflective of the current U.S. adult population. Furthermore, the MSA data was self-reported over the past 30 days and not objectively measured. As a result, the frequency, intensity, duration, and type of MSA is subject to recall bias and possible social desirability effect. Lastly, due to the nature of the cross-sectional study design, causality will not be able to be established.

**Summary**

The three research questions in this study were: 1) “Is there an association between volumes of MSA meeting the current DHHS recommendation and having an elevated CRP level?”, 2) “If a relationship does exist, is that relationship different for each gender?”, and 3) “Does waist circumference mediate this relationship.”

This is the first study to examine the potential gender differences in elevated CRP levels and volumes of MSA in adults aged ≥20 years in a nationally representative

sample of U.S. adults. This study adds to the evidence demonstrating gender specific differences between volumes of MSA and markers of inflammation.

**References**


1. Physical activity guidelines for Americans; Be active, healthy, and happy. United States Department of Health and Human Services 2008.
2. National Center for Health Statistics Centers for Disease Control and Prevention. NHANES Analytic and Reporting Guidelines. September 2006 version.
3. SAS. Base SAS 9.2 Procedures Guide. Carey, NC: 2009.
4. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003;107:499-511.
5. Laboratory Procedure Manual: C-Reactive Protein in Serum NHANES 2003–2004.
6. Benowitz NL, Bernert JT, Caraballo RS, Holiday DB, Wang J. Optimal serum cotinine levels for distinguishing cigarette smokers and nonsmokers within different racial/ethnic groups in the United States between 1999 and 2004. Am J Epidemiol 2009;169:236-48.
7. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA 2001;285:2486-97.
8. Hirsch GA, Vaid N, Blumenthal RS. The Significance of Measuring Non-HDL-Cholesterol. Preventive cardiology 2002;5:156-159.

9. Prasad K. C-reactive protein (CRP)-lowering agents. Cardiovasc Drug Rev 2006;24:33-50.

10. American Diabetes A. Diagnosis and classification of diabetes mellitus. Diabetes care 33:S62-S69.

11. Research Triangle Institute. SUDAAN User's Manual, Release 8.0. Research Triangle Park, NC: 2001.

# Chapter Four: Gender Differences in High Sensitivity C - reactive Protein and Self-Reported Muscle Strengthening Activity among U.S. Adults

**Objectives** We sought to examine the gender differences between C - reactive protein (CRP) and muscle strengthening activity (MSA) in U.S. adults (≥20 years of age).

**Background** Elevated levels of CRP have been shown to be associated with an increase in risk of cardiovascular disease (CVD). Studies analyzing the relationship between physical activity (PA) and CRP by gender have produced mixed results.

**Methods** The sample (n=9,135) included participants in the 1999-2004 National Health and Nutrition Examination Survey (NHANES). Three categories of reported MSA participation were created: no MSA (referent group), some MSA (≥1 to <2 d/wk), and meeting the 2008 Department of Health and Human Services (DHHS) recommendation (≥2 d/wk). The dependent variable was elevated CRP (>3 to 10 mg/L).

**Results** Gender stratified analysis revealed significantly lower odds of having elevated CRP for women reporting some MSA (OR 0.61; 95% CI 0.45-0.83, P=0.0023), or volumes of MSA meeting the DHHS recommendation (OR 0.66; 95% CI 0.54-0.82, P=0.0004). Significantly lower odds of men having elevated CRP was observed in those reporting MSA volumes meeting the recommendation (OR 0.73; 95% CI 0.61-0.88, P=0.0011). Following adjustment for waist circumference (WC) these odds remained significant in men but not women.

**Conclusions** Women reporting any MSA were found to have lower odds of having elevated CRP when compared to those reporting no MSA prior to

adjustment for WC. Significantly lower odds in men were only observed in those meeting the recommendation. These results suggest that WC may mediate the associations between MSA and CRP and this relationship may be stronger in women.

Elevated levels of C - reactive protein (CRP), a non-specific marker of inflammation, have been shown to be associated with an increase in risk of cardiovascular disease (CVD) (1). Moreover, previous cross-sectional analyses have revealed statistically significant positive associations between CRP concentrations and self-reported myocardial infarction (2) and stroke (3). It has also been shown that physical activity (PA) participation has a role in reducing incidence of CVD (4). Although PA may produce a short term rise in circulating levels of CRP, long term participation in exercise training has been shown to reduce CRP levels (5). Some studies have suggested that the associations between PA and CVD may be mediated by inflammation (6,7).

The Department of Health and Human Services (DHHS) 2008 Physical Activity Guidelines for Americans recommend that adults should do muscle-strengthening activities (MSA) that are moderate or high intensity and involve all major muscle groups on two or more d/wk because MSA provides additional benefits not found with aerobic activity (8). The benefits of MSA include increased bone strength, muscular fitness, and help maintaining muscle mass during a program designed to promote weight loss (8). Studies have also shown that protocols utilizing MSA are effective in decreasing circulating levels of CRP (9-11). However, there are few gender-stratified studies analyzing the distribution of elevated CRP (>3 mg/L) or the relationship between PA and CRP, and the limited current literature has revealed mixed results (12-17). The purpose of this study is to examine the associations between self-reported MSA and CRP in adult participants of the National Health and Nutrition Examination Survey (NHANES) when stratified by gender.

**Methods**

This study utilized six years of data from the 1999-2004 NHANES, a continuous survey conducted by the National Center for Health Statistics (18). The NHANES is designed to provide national estimates of the health and nutritional status of non-institutionalized United States (U.S.) civilians over the age of two months. The overall response rates ranged from 76% to 80% for participants selected for examination in the 1999-2004 NHANES. The total 1999-2004 NHANES sample size was 31,126, ages two months and above. A total of 15,332 adults (≥20 years of age) provided responses to the interview portion of 1999-2004 NHANES. Following the exclusion of 833 pregnant women and 16 adults with missing responses related to MSA participation a total of 14,483 participants were available for further analysis. Of these participants, 12,643 adults (87%) attended a mobile examination center and provided the serum necessary for the measurement of CRP. Following the exclusion of adults with any missing values for other study covariates, the final sample consisted of 4,079 male and 5,056 female U.S. adults.

The primary independent variable in this study was frequency of participation in MSA activities and was calculated from 'self-reported' MSA patterns. The final sample provided responses to the following items which came from the physical activity questionnaire file item PAD440: *Over the past 30 days, did {you/SP} do any physical activities specifically designed to strengthen {your/his/her} muscles such as lifting weights, push-ups or sit-ups? Include all such activities even if you have mentioned them before in the past 12 months.* The sample also provided responses to PA questionnaire file item PAD460: *Over the past 30 days, how often did you do these activities? [Activities designed to strengthen {your/his/her} muscles such as lifting weights, push-*

*ups or sit-ups.]* The MSA variable was created with three categories: no MSA, some MSA, and meeting the DHHS recommendations. No MSA was coded as 0 d/wk, some MSA as ≥1 to <2 d/wk, and meeting the recommendation as ≥2 d/wk.

Four categories of race were created: non-Hispanic white, non-Hispanic black, Mexican American, and other. Serum cotinine, a metabolite of nicotine that is used as a marker for active smoking and environmental tobacco smoke exposure, was dichotomized (yes/no) based on having a serum level ≥3 ng/mL (19). Non-high density lipoprotein cholesterol (non-HDL-C) was calculated by subtracting HDL-C from total cholesterol and divided into four categories: <130, 130 to <160, 160 to <190, and ≥190 mg/dL based on goals recommended in the Third Report of the National Education Program Adult Treatment Panel III (NCEP ATP III) (20). Reduced HDL-C, based on the NCEP ATP III and American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) definitions, was dichotomized (yes/no) for men (<40 mg/dL or drug treatment) and women (<50 mg/dL or drug treatment) (20,21). Glycohemoglobin (HbA1c %) was divided into three categories based on American Diabetes Association (ADA) recommendations for diagnosis of diabetes mellitus: <5.7%, 5.7 to 6.4%, and >6.4% (22). Hypertension (systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or current antihypertensive drug treatment) was dichotomized (yes/no) using the NCEP ATP III definition (20). A dichotomous hormone therapy variable (yes/no) was created for women based on responses to the following item from the reproductive health questionnaire file item RHQ540: *{Have you/Has SP} ever used female hormones such as estrogen and progesterone? Please include any forms of female hormones, such as pills, cream, patch, and injectables, but do not include birth control*

*methods or use for fertility*. Lastly, waist circumference (WC), which was measured using steel tape just above the uppermost lateral border of the right ilium to the nearest millimeter, was examined continuously in order to examine the odds of having an elevated CRP concentration for every centimeter increment of WC.

The dependent variable in this study was elevated CRP (>3 to10 mg/L) (23). Tests with a CRP level of >10 mg/L are often due to acute conditions, and recommendations are to exclude such tests (23). C - reactive protein concentrations were measured using latex-enhanced nephelometry (Dade Behring Nephelometer II Analyzer System) at the University of Washington Medical Center. Seattle, Washington.

The data in this study were initially managed using SAS 9.2 (24). SAS was used to conduct both complex variable recodes and data coding validation. SAS-callable SUDAAN (25) was then used to conduct the analysis, incorporating sampling weights within the context of the complex multistage sampling design inherent to NHANES. Following a forward selection process based on Wald F-test results, gender-specific best fit models were constructed. The resultant logistic regression models for men included the following covariates: HDL-C, non-HDL-C, HbA1c %, hypertension, serum cotinine, and WC. Best fit logistic regression models for women included the following covariates: race, non-HDL-C, hormone therapy, and WC. Possible effect modification by gender or MSA participation was tested by adding an interaction term to the logistic regression models.

## Results

Table 1 illustrates the prevalence estimates for elevated CRP in U.S. adults according to sample characteristics.

**Table 1. Prevalence of Elevated CRP According to Sample Characteristics: NHANES 1999-2004**

| *Covariates* | **Men** | | **Women** | |
|---|---|---|---|---|
| | *N* | *Weighted % (SE %)* | *N* | *Weighted % (SE %)* |
| **Total** | 4072 | 24.4 (0.93) | 3464 | 36.0 (0.91) |
| **MSA** | | | | |
| None | 2977 | 26.7 (1.08) | 2756 | 38.5 (1.14) |
| Some Activity | 248 | 20.4 (3.15) | 172 | 25.5 (2.62) |
| Meets Recommendation | 847 | 19.8 (1.30) | 536 | 30.3 (2.15) |
| **Age** | | | | |
| 20-39 | 1265 | 18.9 (1.31) | 1025 | 30.3 (1.60) |
| 40-59 | 1302 | 25.8 (1.43) | 1070 | 38.4 (1.61) |
| ≥ 60 | 1505 | 31.4 (1.51) | 1369 | 42.0 (1.83) |
| **Race** | | | | |
| non-Hispanic white | 2140 | 23.8 (1.14) | 1790 | 35.3 (1.15) |
| non-Hispanic black | 687 | 27.7 (1.69) | 597 | 38.6 (2.31) |
| Mexican American | 962 | 23.8 (1.21) | 801 | 44.3 (1.60) |
| Other | 283 | 26.1 (2.51) | 276 | 34.6 (2.92) |
| **Education** | | | | |
| < High School | 1372 | 29.1 (1.65) | 1096 | 39.7 (2.28) |
| High School Graduate | 937 | 25.1 (1.55) | 850 | 36.8 (1.78) |
| > High School | 1763 | 22.4 (1.17) | 1518 | 35.0 (1.55) |
| **Cotinine (ng/mL)** | | | | |
| < 3 | 2798 | 22.6 (1.17) | 2754 | 35.8 (1.03) |
| ≥ 3 | 1274 | 28.3 (1.49) | 710 | 36.6 (1.83) |
| **Low HDL-C (mg/dL)** | | | | |
| No | 2551 | 20.7 (1.04) | 1965 | 31.6 (1.11) |
| Yes | 1521 | 30.6 (1.61) | 1499 | 42.9 (1.46) |
| **Non-HDL-C (mg/dL)** | | | | |
| < 130 | 1370 | 20.2 (1.34) | 1361 | 28.6 (1.36) |
| 130 to < 160 | 1113 | 22.6 (1.12) | 926 | 37.4 (1.57) |
| 160 to < 190 | 886 | 27.3 (2.05) | 670 | 41.4 (2.40) |
| ≥ 190 | 703 | 31.5 (2.33) | 507 | 52.3 (2.87) |
| **Glycohemoglobin (%)** | | | | |
| < 5.7 | 2957 | 21.8 (0.88) | 2585 | 33.5 (1.05) |
| 5.7 to < 6.5 | 707 | 38.0 (3.58) | 590 | 57.9 (4.18) |
| ≥ 6.5 | 408 | 41.1 (5.38) | 289 | 53.0 (7.40) |
| **Hypertension** | | | | |
| SBP < 140 and DBP < 90 | 2582 | 22.1 (1.22) | 2120 | 33.3 (1.03) |
| SBP ≥ 140, or DBP ≥ 90 | 1490 | 32.5 (1.99) | 1344 | 43.1 (3.15) |
| **Augmented WC** | | | | |
| No | 2437 | 17.1 (1.02) | 1367 | 20.8 (1.22) |
| Yes | 1635 | 36.4 (1.95) | 2097 | 50.4 (1.23) |
| **Hormone Therapy** | | | | |
| No | | | 2473 | 33.8 (1.16) |
| Yes | | | 991 | 41.8 (3.82) |

Independent variables included muscle strengthening activity (MSA), age (years), race, education, serum cotinine, low high-density lipoprotein cholesterol (HDL-C) Men (<40 mg/dL) Women (<50 mg/dL), non-high-density lipoprotein cholesterol (non-HDL-C), Glycohemoglobin (%), hypertension, augmented waist circumference (WC) Men (yes: ≥102 cm, no: <102 cm), Women (yes: ≥88 cm, no <88 cm), and hormone therapy.

Abbreviations: Elevated CRP, elevated C - reactive protein (>3 mg/L to ≤10 mg/L); SE, standard error; SBP, systolic blood pressure; DBP, diastolic blood pressure; ng/mL, nanogram per milliliter; mg/dL, milligram per deciliter

The age-adjusted prevalence of elevated CRP levels among U.S. adult men and women was 24.4% and 36.0%, respectively (Table 1). The prevalence of elevated CRP increased with age in both men and women and was higher among Mexican-Americans and non-Hispanic blacks. Prevalence of elevated CRP was also higher in those with serum cotinine concentrations >3 ng/mL. When examined by non-HDL-C concentration, the prevalence of elevated CRP was shown to increase with increasing non-HDL-C concentration. The adjusted prevalence of elevated CRP concentrations was also increased in those with glycohemoglobin range from 5.7 to <6.5%, or ≥6.5%. The prevalence of elevated CRP was also increased in men and women with an augmented WC, 36.4% and 50.4%, respectively.

The interaction term for gender and MSA participation in the multiple logistic regression model was not significant (P=0.3063), thus the term was not included in the resultant models. Tables 2 and 3 illustrate the results of the logistic regression analyses examining the associations between elevated CRP concentrations and MSA.

**Table 2. Odds Ratios for Muscle Strengthening Activity as a Predictor of Elevated CRP for Men in a Sample from the National Health and Nutrition Examination Survey Cycles 1999–2004**

| *Variable* | *Model 1 OR (95% CI)* | *Model 2 OR (95% CI)* | *Model 3 OR (95% CI)* |
|---|---|---|---|
| **MSA** | | | |
| None | 1.00 | 1.00 | 1.00 |
| Some Activity | 0.65 (0.46-0.92)* | 0.80 (0.55-1.17) | 0.90 (0.61-1.34) |
| Meets Recommendation | 0.57 (0.49-0.67)* | 0.73 (0.61-0.88)* | 0.77 (0.63-0.95)* |
| **Non-HDL-C (mg/dL)** | | | |
| < 130 | | 1.00 | 1.00 |
| 130 to < 160 | | 1.20 (0.96-1.50) | 1.11 (0.88-1.41) |
| 160 to < 190 | | 1.59 (1.19-2.12)* | 1.43 (1.07-1.91)* |
| ≥ 190 | | 1.78 (1.36-2.31)* | 1.56 (1.19-2.04)* |
| **HDL-C (mg/dL)** | | | |
| ≥ 40 | | 1.00 | 1.00 |
| < 40 | | 1.50 (1.25-1.81)* | 1.24 (1.02-1.51)* |
| **Cotinine (ng/mL)** | | | |
| < 3 | | 1.00 | 1.00 |
| ≥ 3 | | 1.31 (1.09-1.59)* | 1.55 (1.25-1.94)* |
| **Glycohemoglobin (%)** | | | |
| < 5.7 | | 1.00 | 1.00 |
| 5.7 to < 6.5 | | 1.79 (1.48-2.16)* | 1.43 (1.14-1.78)* |
| ≥ 6.5 | | 1.84 (1.27-2.66)* | 1.35 (0.94-1.93) |
| **Hypertension** | | | |
| SBP ≤ 140 and DBP ≤ 90 | | 1.00 | 1.00 |
| SBP > 140, or DBP > 90 | | 1.67 (1.32-2.12)* | 1.32 (1.02-1.71)* |
| **WC (cm)** | | | |
| | | | 1.04 (1.03-1.05)* |

Independent variable(s) included in Model 1: muscle strengthening activity; Model 2: muscle strengthening activity, non-high density lipoprotein cholesterol, high density lipoprotein cholesterol, Serum Cotinine, Glycohemoglobin, and hypertension; Model 3 included all variables from Model 2 and waist circumference.

*Significant predictors ($P$<0.05).

Abbreviations: OR, odds ratio; CI, confidence interval; MSA, muscle strengthening activity; HDL-C, high density lipoprotein cholesterol; WC, waist circumference; BP, blood pressure; SBP, systolic blood pressure; DBP; diastolic blood pressure; cm, centimeter; mg/dL, milligram per deciliter; ng/mL, nanogram per milliliter

**Table 3. Odds Ratios for Muscle Strengthening Activity as a Predictor of Elevated CRP for Women in a Sample from the National Health and Nutrition Examination Survey Cycles 1999–2004**

| *Variable* | *Model 1* *OR (95% CI)* | *Model 2* *OR (95% CI)* | *Model 3* *OR (95% CI)* |
|---|---|---|---|
| **MSA** | | | |
| None | 1.00 | 1.00 | 1.00 |
| Some Activity | 0.49 (0.36-0.67)* | 0.61 (0.45-0.83)* | 0.79 (0.56-1.12) |
| Meets Recommendation | 0.62 (0.51-0.75)* | 0.66 (0.54-0.82)* | 0.93 (0.73-1.17) |
| **Non-HDL-C (mg/dL)** | | | |
| < 130 | | 1.00 | 1.00 |
| 130 to < 160 | | 1.48 (1.25-1.75)* | 1.43 (1.18-1.73)* |
| 160 to < 190 | | 1.77 (1.40-2.24)* | 1.53 (1.19-1.96)* |
| ≥ 190 | | 2.69 (2.12-3.43)* | 2.16 (1.70-2.73)* |
| **Race** | | | |
| non-Hispanic white | | 1.00 | 1.00 |
| non-Hispanic black | | 1.23 (1.02-1.49)* | 0.97 (0.80-1.17) |
| Mexican American | | 1.55 (1.25-1.92)* | 1.50 (1.25-1.80)* |
| Other | | 0.92 (0.72-1.17) | 1.05 (0.78-1.40) |
| **Hormone Therapy** | | | |
| No | | 1.00 | 1.00 |
| Yes | | 1.60 (1.35-1.90)* | 1.51 (1.26-1.81)* |
| **WC (cm)** | | | |
| | | | 1.05 (1.04-1.06)* |

Independent variable(s) included in Model 1: muscle strengthening activity; Model 2: muscle strengthening activity, non-high density lipoprotein cholesterol, race, and hormone therapy; Model 3 included all variables from Model 2 and waist circumference.
*Significant predictors ($P$<0.05).
Abbreviations: OR, odds ratio; CI, confidence interval; MSA, muscle strengthening activity; HDL-C, high density lipoprotein cholesterol; WC, waist circumference; cm, centimeter; mg/dL, milligram per deciliter

Crude analysis revealed significantly lower odds of having an elevated CRP level for male participants reporting some MSA (OR 0.65; CI 0.46-0.92, P=0.0162), or volumes meeting the DHHS recommendation (OR 0.57; 0.49-0.67, P<0.0001) when compared to a referent group reporting no MSA (Table 2). Crude analysis also revealed significantly lower odds of having an elevated CRP level for female participants reporting some MSA (OR 0.49; CI 0.36-0.67, P<0.0001), or volumes meeting the DHHS recommendation (OR 0.62; CI 0.51-0.75, P<0.0001) when compared to a referent group of females reporting no MSA (Table 3). Following adjustment for select demographic and metabolic risk factors, the odds of having an elevated CRP level were 27% and 34% lower (P<0.01) in male and female U.S. adults meeting the DHHS recommendation,

respectively (Tables 2 & 3, Model 2). Following adjustment for WC these odds were no longer statistically significant in women (Table 3, Model 3). In contrast, the attenuated odds of elevated CRP remained statistically significant in those reporting volumes of MSA meeting the DHHS recommendation (OR 0.77; CI 0.63-0.95, P=0.0148) (Table 2, Model 3).

Compared to their respective referent groups with non-HDL-C levels <130 mg/dL, men and women with non-HDL-C levels 160 to <190 mg/dL, or ≥190 mg/dL, were significantly more likely (P<0.05, all models) to have an elevated CRP level. However, the increased odds of having an elevated CRP level in men with non-HDL-C concentrations 130 to <160 mg/dL were not statistically significant. Men with low HDL-C concentrations (<40 mg/dL) or elevated serum cotinine concentrations (≥3 ng/mL) were significantly more likely to have an elevated CRP level (P<0.05, all models). Having an HbA1c concentration of 5.7 to 6.4% or hypertension was also associated with a statistically significant increase in odds of having an elevated CRP level in male participants (P<0.05, all models).

Our study findings revealed mixed results when examining the relationships between race and CRP in women. In comparison to a referent group of non-Hispanic white women, the odds of having an elevated CRP level for Mexican American women was significantly higher (P<0.001, all models). In comparison to female participants that did not report using hormone therapies, the odds of having an elevated CRP were significantly higher (P<0.0001, all models) for women using hormone therapies. Lastly, when examining WC measures in men and women the odds of having an elevated CRP

level were significantly higher for every centimeter increment in WC [men (OR 1.04; 95% CI 1.03-1.05, P<0.0001), women (OR 1.05; 95% CI 1.04-1.06, P<0.0001)].

**Discussion**

Although some studies have shown that participation in various forms of MSA is associated with a decrease in concentrations of CRP, the study populations were generally small and not representative of the U.S. population (9-11). For example, in a sample of 44 young male African American and white volunteers, six weeks of resistance training resulted in a significant decrease in CRP in African American subjects (Mean ± SE; Baseline CRP 4.84 ± 0.9 vs. Post Training CRP 2.34 ± 0.5 mg/L, P<0.05) (9). Volunteers trained three d/wk for approximately 60 minutes per session. Three sets of five different exercises per session were performed with a one to two minute rest period between each set. Load was progressively increased to ensure fatigue within approximately eight to12 repetitions. The study protocol utilized a split routine designed to alternate exercises for legs, back, and biceps on one day and exercises for the chest, shoulders, and triceps on a separate day. Thus, the selected exercises would effectively stress the major muscle groups of the upper and lower body. Interestingly, the observed decrease in CRP in these male African American volunteers remained statistically significant following a four week detraining period (CRP 1.91 ± 0.4 mg/L).

Stewart et al. (10) examined the influence of a 12 week exercise protocol that utilized a combination of aerobic and resistance training on CRP concentrations in 29 younger (18-35yr) and 31 older (65-85yr) subjects. Participants trained three days per week and each session included a warm-up, 20 minutes of aerobic training

(walking/jogging) on a treadmill (70-80% of heart rate reserve), and two sets of eight resistance exercises [70-80% one repetition maximum (1RM), second set performed to momentary failure] followed by a stretching and cool down period. Intensity was adjusted bi-weekly. These investigators reported a 58% decrease ($P<0.01$) in serum CRP concentrations from pre- to post-training in those classified as inactive prior to study participation.

Donges et al. (11) investigated the effects of 10 weeks of exercise training on interleukin-6 and CRP in a sample of 102 male (n=45) and female (n=57) sedentary subjects. Subjects were assigned to a resistance group (n=35), an aerobic group (n=41), or a control group (n=26) that remained sedentary. Training sessions began with five minutes of dynamic stretching and concluded with five minutes of stretching. The resistance training protocol utilized pulley-weight machines and exercises were performed at 10 repetition maximum (~75% of a 1RM). Resistance exercises included chest press, shoulder press, lat pull-down, seated row, leg press, leg curl, and lunge. Participants performed two to three sets of eight to10 repetitions with a two-minute rest period between sets. Intensity was adjusted accordingly to promote training to ‘‘momentary muscle failure.’’ Aerobic training (~75% of maximum heart rate) was conducted using Monark stationary cycle ergometers. Interestingly, only resistance training resulted in a significant attenuation of CRP concentration, 32.8% ($P<0.05$).

Our findings support previous studies reporting adiposity as a mediating factor in the association between MSA and CRP levels in men and women (26-31). In an analysis using baseline data from the Health, Aging and Body Composition Study, Colbert et al. (27) reported that CRP levels were significantly lower in older (70-79 yr) black and white

adult study participants reporting higher levels of exercise (≥180 min/wk) that included resistance training (e.g., weight training, calisthenics). However, the relationship between exercisers and non-exercisers was not statistically significant following adjustments for total body fat and visceral fat. Other studies have also noted the associations between body fat and elevated CRP concentrations (28), an association which has also been consistently shown to be stronger in women (26,29-31).

When stratified by gender, previous studies analyzing the distribution of elevated CRP (>3 mg/L), or the relationship between PA and CRP, have produced mixed results (12-17). In a study of older adults conducted by Canon et al. (14), elevated levels of CRP mediated the relationship between cognitive function and muscle quality in females but not males. Albert et al. (13) reported that concentrations of CRP in participants of the Pravastatin Inflammation/CRP Evaluation study were statistically significantly lower among middle-aged men who self-reported a high level (≥4 times/wk) of strenuous aerobic PA (e.g., swimming, running, aerobics, cycling). However, when compared with a referent group of reporting participating in strenuous aerobic PA less than once per week, this relationship was not statistically significant in women (mean age in years, 60.8 ± 12 for both men and women). A study examining the association between fitness and CRP levels in children and young adult participants (n=205) in the Columbia University BioMarkers Study aged six to 24 years revealed statistically significant inverse associations only in male participants (12). Previous studies have also demonstrated independent associations between CRP and hand grip and lower body strength that appear to be stronger in women (32,33).

In men, the 1999-2004 NHANES data showed a statistically significant inverse association between having an elevated CRP concentration and meeting the current DHHS recommendations. In women, a statistically significant inverse association was demonstrated in those reporting any level of MSA participation. These associations were independent of race, increased levels of non-HDL-C, and hormone therapies in women and reduced HDL-C levels, increased levels of non-HDL-C, increased HbA1c, hypertension, and increased serum cotinine concentrations in men. However, adjustment for WC attenuated the protective association between reporting MSA and having elevated CRP in men and resulted in an absence of statistically significant associations in women for any volume of MSA. Since CRP has shown efficacy in predicting risk of incident CVD (34), these findings may be particularly important for men and women who do not currently perform any volume of MSA, or for men performing volumes of MSA that do not meet the recommendation.

The observed associations between CRP and MSA were also consistent with other studies which have demonstrated more pronounced associations among non-Caucasian female subgroups (15,16) and those reporting adiposity as a strong mediating factor (17,28,29). Consequently, because MSA participation has been estimated to be lower in female subgroups (35), it may become increasingly important for health care professionals to promote MSA participation in these groups.

Our study included some inherent strengths and limitations. The NHANES sample is large and representative of the U.S adult population. This strengthens the external validity of our study. However, since the data for these analyses were collected from 1999-2004 it may not be reflective of the current population. Other limitations

include the use of the MSA data, which were self-reported over the past 30 days and not objectively measured. Thus, the frequency, intensity, duration, and type of MSA are subject to recall bias. Lastly, due to the nature of the cross-sectional study design, causality cannot be inferred.

## Conclusions

Our population-based findings add to the current evidence suggesting that MSA is associated with lower levels of CRP. Women reporting any MSA were found to have significantly lower odds of having elevated CRP when compared to those reporting no MSA. However, significantly lower odds in men were only observed in those meeting the DHHS recommendation. These results also suggest that adiposity may mediate the associations between MSA and CRP. Future studies should examine the associations among MSA, CRP, and other markers of metabolic health.

**References**

1. Kaptoge S, Di Angelantonio E, Lowe G, et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet 2010;375:132-40.
2. Ford ES, Giles WH. Serum C-reactive protein and fibrinogen concentrations and self-reported angina pectoris and myocardial infarction: findings from National Health and Nutrition Examination Survey III. J Clin Epidemiol 2000;53:95-102.
3. Ford ES, Giles WH. Serum C-reactive protein and self-reported stroke: findings from the Third National Health and Nutrition Examination Survey. Arterioscler Thromb Vasc Biol 2000;20:1052-6.
4. Nocon M, Hiemann T, Muller-Riemenschneider F, Thalau F, Roll S, Willich SN. Association of physical activity with all-cause and cardiovascular mortality: a systematic review and meta-analysis. Eur J Cardiovasc Prev Rehabil 2008;15:239-46.
5. Kasapis C, Thompson PD. The effects of physical activity on serum C-reactive protein and inflammatory markers: a systematic review. J Am Coll Cardiol 2005;45:1563-9.
6. Mora S, Cook N, Buring JE, Ridker PM, Lee IM. Physical activity and reduced risk of cardiovascular events: potential mediating mechanisms. Circulation 2007;116:2110-8.
7. Wannamethee SG, Lowe GD, Whincup PH, Rumley A, Walker M, Lennon L. Physical activity and hemostatic and inflammatory variables in elderly men. Circulation 2002;105:1785-90.

8. Physical activity guidelines for Americans; Be active, healthy, and happy. United States Department of Health and Human Services 2008.

9. Heffernan KS, Jae SY, Vieira VJ, et al. C-reactive protein and cardiac vagal activity following resistance exercise training in young African-American and white men. Am J Physiol Regul Integr Comp Physiol 2009;296:R1098-105.

10. Stewart LK, Flynn MG, Campbell WW, et al. The influence of exercise training on inflammatory cytokines and C-reactive protein. Med Sci Sports Exerc 2007;39:1714-9.

11. Donges CE, Duffield R, Drinkwater EJ. Effects of resistance or aerobic exercise training on interleukin-6, C-reactive protein, and body composition. Med Sci Sports Exerc 2010;42:304-13.

12. Isasi CR, Deckelbaum RJ, Tracy RP, Starc TJ, Berglund L, Shea S. Physical fitness and C-reactive protein level in children and young adults: the Columbia University BioMarkers Study. Pediatrics 2003;111:332-8.

13. Albert MA, Glynn RJ, Ridker PM. Effect of physical activity on serum C-reactive protein. Am J Cardiol 2004;93:221-5.

14. Canon ME, Crimmins EM. Sex differences in the association between muscle quality, inflammatory markers, and cognitive decline. J Nutr Health Aging 2011;15:695-8.

15. Majka DS, Chang RW, Vu TH, et al. Physical activity and high-sensitivity C-reactive protein: the multi-ethnic study of atherosclerosis. Am J Prev Med 2009;36:56-62.

16. Khera A, McGuire DK, Murphy SA, et al. Race and gender differences in C-reactive protein levels. J Am Coll Cardiol 2005;46:464-9.

17. Geffken DF, Cushman M, Burke GL, Polak JF, Sakkinen PA, Tracy RP. Association between physical activity and markers of inflammation in a healthy elderly population. Am J Epidemiol 2001;153:242-50.

18. National Center for Health Statistics Centers for Disease Control and Prevention. NHANES Analytic and Reporting Guidelines. September 2006 version.

19. Benowitz NL, Bernert JT, Caraballo RS, Holiday DB, Wang J. Optimal serum cotinine levels for distinguishing cigarette smokers and nonsmokers within different racial/ethnic groups in the United States between 1999 and 2004. Am J Epidemiol 2009;169:236-48.

20. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA 2001;285:2486-97.

21. Grundy SM, Cleeman JI, Daniels SR, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 2005;112:2735-52.

22. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes care 2010;33:S62-S69.

23. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A

statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003;107:499-511.

24. SAS. Base SAS 9.2 Procedures Guide. Carey, NC: 2009.

25. Research Triangle Institute. SUDAAN User's Manual, Release 8.0. Research Triangle Park, NC: 2001.

26. Festa A, D'Agostino R, Jr., Williams K, et al. The relation of body fat mass and distribution to markers of chronic inflammation. Int J Obes Relat Metab Disord 2001;25:1407-15.

27. Colbert LH, Visser M, Simonsick EM, et al. Physical activity, exercise, and inflammatory markers in older adults: findings from the Health, Aging and Body Composition Study. J Am Geriatr Soc 2004;52:1098-104.

28. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-reactive protein levels in overweight and obese adults. JAMA 1999;282:2131-5.

29. Thorand B, Baumert J, Doring A, et al. Sex differences in the relation of body composition to markers of inflammation. Atherosclerosis 2006;184:216-24.

30. Connelly PW, Hanley AJ, Harris SB, Hegele RA, Zinman B. Relation of waist circumference and glycemic status to C-reactive protein in the Sandy Lake Oji-Cree. Int J Obes Relat Metab Disord 2003;27:347-54.

31. Lear SA, Chen MM, Birmingham CL, Frohlich JJ. The relationship between simple anthropometric indices and C-reactive protein: ethnic and gender differences. Metabolism 2003;52:1542-6.

32. Hamer M, Molloy GJ. Association of C-reactive protein and muscle strength in the English Longitudinal Study of Ageing. Age Dordr 2009;31:171-7.

33. Visser M, Pahor M, Taaffe DR, et al. Relationship of interleukin-6 and tumor necrosis factor-alpha with muscle mass and muscle strength in elderly men and women: the Health ABC Study. J Gerontol A Biol Sci Med Sci 2002;57:M326-32.

34. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation 2003;107:363-9.

35. Carlson SA, Fulton JE, Schoenborn CA, Loustalot F. Trend and prevalence estimates based on the 2008 Physical Activity Guidelines for Americans. Am J Prev Med 2010;39:305-13.

# Appendices

# Appendix A

UNF
UNIVERSITY of NORTH FLORIDA

Office of Research and Sponsored Programs
1 UNF Drive
Jacksonville, FL 32224-2665
904-620-2455 FAX 904-620-2457
Equal Opportunity/Equal Access/Affirmative Action Institution

**MEMORANDUM**

**DATE:** February 7, 2014

**TO:** Mr. Michael Richardson

**FACULTY:** Dr. James Churilla
Clinical and Applied Movement Sciences

**FROM:** Dr. Jennifer Wesely
On behalf of the UNF Institutional Review Board

**RE:** Project outline review on behalf of the UNF Institutional Review Board IRB

---

This is to advise you that your projects, as outlined below, were discussed and reviewed on behalf of the UNF Institutional Review Board. Projects as outlined are declared "not human subjects research" based on the federal definition of "research" as stated in the U.S. Department of Health and Human Services Code of Federal Regulations 45 Part 46. Therefore, it is not necessary for projects fitting the description below to be reviewed and approved by the UNF IRB.

Based on conversations with the Dr. Churilla, UNF's IRB understands that Mr. Richardson works with publicly available data from CDC's NHANES (National Health and Nutrition Exam Survey) and conducts secondary data analysis. All data sets received are fairly large and de-identified. Although the PI conducts systematic investigations with the intent to generalize and the information obtained is about living individuals, the PI neither intervenes nor interacts with the individuals. Therefore, this work is not human subject research and is not subject to 45 CFR 46. An IRB submission and review is not necessary.

This waiver should be kept for your records and applies to your project in the form and content as submitted to the IRB for review. Any variations or modifications to waived projects as related to dealing with human subjects must be cleared with the IRB prior to implementing such changes. Any unanticipated problems involving risk and any occurrence of serious harm to subjects and others shall be reported promptly to the IRB.

Thank you for submitting your work for IRB review. We appreciate that you understand the value of IRB review of human subject research conducted at UNF.

Should you have any questions or if we can be of further service, please contact Office of Research and Sponsored Programs

Research Integrity Staff

# Appendix B

## Associations between High Sensitivity C - reactive Protein and Self-Reported Screen Time in U.S. Adults

**Objectives** The purpose of this study was to examine the associations between elevated CRP and screen time in a nationally representative sample of U.S. adults.

**Background** High sensitivity C - reactive protein (CRP) has shown efficacy in predicting risk of adverse cardiovascular events and increased screen time has been shown to be positively associated with cardiometabolic risk.

**Methods** Study sample (n=7,645) included adults (≥20 years of age) who participated in the 2003-2006 National Health and Nutrition Examination Survey. Logistic regression was utilized to examine the association between elevated CRP and screen time.

**Results** Analysis revealed that individuals with CRP concentrations >3 to10 mg/L had an increased odds of reporting ≥3 hours/day of screen time when compared to those with CRP levels <3 mg/L (OR 1.27; 95% CI 1.15-1.41, P<0.001). This association remained significant (OR 1.25; 95% CI 1.13-1.39, P<0.001) following adjustment for physical activity and waist circumference.

**Conclusions** These data suggest that elevated levels of CRP were significantly associated with reporting greater amounts of self-reported screen time independent of meeting physical activity recommendations or waist circumference.

C - reactive protein (CRP) is an acute phase protein synthesized by hepatocytes and adipocytes in response to inflammation (1). C - reactive protein has shown usefulness as a predictor of the future risk of adverse vascular events (2). In 2003 a statement for healthcare professionals from the Centers for Disease Control and Prevention (CDC) and the American Heart Association (AHA) on the clinical application of markers of inflammation and cardiovascular disease (CVD) (3) was issued suggesting the use of CRP as a risk marker for CVD in those with Framingham risk scores projecting a 10 year coronary heart disease (CHD) risk between 10% and 20%. The AHA and the CDC designate CRP levels by relative risk categories, with low, average, and high corresponding to approximate values of <1.0, 1.0-3.0, and >3.0 mg/L.

Findings from studies using data from the 1999-2000 National Health and Nutrition Examination Survey (NHANES) have been used to provide prevalence estimates of CRP values ≥ 3 mg/L in adult (≥20 yr) men (4) and women (5). The findings from these studies revealed that 22.1% of men (n=1,940) and 30.6% of women not currently taking hormone replacement (n=1,787) had elevated CRP concentrations (3<CRP≤10 mg/L). Furthermore, multivariable logistic regression analysis comparing these age-adjusted proportions has revealed significantly increased odds of having an elevated CRP concentration in women when compared with men (OR 2.59; 95% CI 1.96–3.42, P<0.001).

According to the Nielsen Cross-Platform Report (6), Americans spent approximately 34 hours per week viewing television in the third quarter of 2012. It was also reported that Americans are spending close to five hours per week viewing a computer screen. Television was by far the most predominant form of screen time (ST)

being reported. Analysis of prospective studies suggest that longer durations of ST are consistently associated with a higher risk of diabetes, fatal or nonfatal CVD, and all-cause mortality (7). In the United States (U.S.) population, it has been estimated that 25% of adults are sedentary and 60% are not regularly active (8). An analysis by Katzmarzyk et al. (9) indicates that life expectancy may be extended by two years if time spent sitting was reduced to <3 h/day and 1.38 years if television viewing was reduced to <2 h/day. Studies investigating television viewing and leisure time sedentary behaviors have also found positive correlations among markers of CVD and sedentary behavior independent of physical activity (PA) participation (10-13). Sisson et al. (10) reported an approximated two-fold increase in odds of metabolic syndrome for men reporting ≥4 h/day of ST independent of meeting current PA recommendations.

Accumulating evidence has shown that high sensitivity CRP decreases in a dose response manner with increased amounts of self-reported leisure time PA (14). Consequently, the inverse association is postulated to exist with the absence of PA. The purpose of this study was to examine the relationship between self-reported ST, using the 2003-2006 NHANES questionnaire data collected on the hours spent viewing television and using a computer, and CRP in a population based sample of U.S. adults. Moreover, we will assess the associations with elevated CRP serving as the exposure variable and the likelihood of reporting increased amounts of screen time as the outcome. To the author's knowledge, the multiple logistics regression model employed in this analysis has never been previously attempted.

**Methods**

This cross-sectional study utilized four years of data from the 2003-2006 NHANES, a continuous survey conducted by the National Center for Health Statistics (15). The NHANES uses complex, multistage probability design to obtain a representative sample of the non-institutionalized U.S. population. The NHANES provides national estimates of the health and nutritional status of U.S. civilians over the age of two months. The total 2003-2006 NHANES sample was 20,470. For this study, the final sample consisted of 7,645 male and female adults (≥20yr). Participants in this study included those who met the following criteria; provided PA data during the household interview; provided non-fasting serum samples necessary for the measurement of CRP in a mobile examination center; if female, not pregnant; and completed an informed consent.

The dependent variable in this study was calculated from 'self-reported' TV and computer use patterns by hours per day. The final sample provided responses to the following items which came from the Physical Activity Questionnaire (PAQ) file; PAD590: *Over the past 30 days, on average about how many hours per day did {you/SP} sit and watch TV or videos?* PAD600: *Over the past 30 days, on average about how many hours per day did {you/SP} use a computer or play computer games?* (16,17). A dichotomized hours of screen time per day variable was created (<3hours/day and ≥3hours/day).

The independent variable of interest in this study was elevated CRP levels (>3 to 10 mg/L). This level corresponds to the CRP level classified as high risk according to the AHA and National Heart, Lung, and Blood Institute recommendations (3). As per the AHA recommendation, those with CRP levels >10 mg/L were excluded from analysis

since these levels are associated with the presence of acute inflammation. The AHA recommends that CRP levels >10 mg/L be discarded and repeated in two weeks to allow acute inflammations to subside before retesting. The created CRP variable was dichotomized (elevated CRP, yes/no). In the 2003-2006 NHANES CRP concentrations were measured using latex enhanced nephelometry on a Dade Behring Nephelometer II Analyzer System at the University of Washington Medical Center. Seattle, Washington (18,19).

Age was divided into three categories: 20-39, 40-59, and ≥60 years. Four categories of race were created: non-Hispanic white, non-Hispanic black, Mexican American, and other. Education was used as the measure of socioeconomic status. Education was divided into four categories: less than high school, high school graduate, some college, and college graduate. A three level smoking status variable was created: current smoker, former smoker, and non-smoker. Augmented waist circumference was dichotomized for men (≥102 cm, yes or no) and women (≥88 cm, yes or no) (20).

The PA variable used in examining the possible inverse associations between volumes of PA and ST was based on the 2007 American College of Sports Medicine/AHA PA recommendation (21). Volumes of PA ($MET \cdot min \cdot wk^{-1}$) were divided into three categories: inactive, insufficiently active, and meets PA recommendations. Individuals reporting five d/wk of moderate intensity PA for 30 minutes, or three d/wk of vigorous intensity PA for 20 minutes, were classified as meeting the recommendation. Individuals reporting at least one time/wk at any duration or intensity were classified in the insufficient category. Individuals reporting no PA were classified as inactive.

Data from the 2003-2006 NHANES were analyzed to determine the odds of U.S. adults with elevated CRP concentrations reporting increased amounts of ST. The data in this study were initially managed using SAS 9.2 (22). SAS was used to conduct complex variable recodes and data coding validation. SAS-callable SUDAAN (23) was used to conduct the analysis, incorporating sampling weights within the context of the correlated multi-stage complex sampling design inherent to NHANES. PROC CROSSTAB was used for calculating descriptive statistics for the categorical variables. Multivariable logistic regression models, using PROC RLOGIST, were created to calculate the odds ratios and 95% confidence intervals for participants who reported increased amounts of ST having elevated CRP. The three logistic regression models that were created included the primary independent variable CRP controlling for age, a second model adding gender, race, education, smoking status, and waist circumference (WC), and a third model controlling for PA volume. Any participants who responded 'don't know/not sure', refused to answer, or had missing responses for any of the questions or measures were excluded from analysis. Less than 10% of survey participants had missing television and computer screen time data. Of the participants that provided serum blood samples in the NHANES mobile examination centers, approximately 10% had missing data for CRP in the 2003-2004 and the 2005-2006 cycles of NHANES.

**Results**

Prevalence estimates revealed that 31.4 % of participants reporting three or more hours of ST had elevated CRP values. In contrast, 24.5% of participants reporting less than three hours had elevated CRP values ($P<0.001$). Table 1 shows the results of the logistic

regression analysis examining the association between elevated CRP and increased amounts of ST.

**Table 1. Odds Ratios for reporting ≥3 hours per day of screen time among adult participants ≥20 years of age: NHANES 2003-2006**

| *Variable* | *Model 1 OR (95% CI)* | *Model 2 OR (95% CI)* | *Model 3 OR (95% CI)* |
|---|---|---|---|
| **CRP** | | | |
| ≤3 mg/L | 1.00 | 1.00 | 1.00 |
| >3 mg/L, ≤10 mg/L | 1.37(1.23-1.53)* | 1.27(1.15-1.41)* | 1.25(1.13-1.39)* |
| **Age** | | | |
| 20-39 | 1.00 | 1.00 | 1.00 |
| 40-59 | 1.00(0.86-1.16) | 0.95(0.81-1.10) | 0.90 (0.78-1.05) |
| ≥60 | 2.07(1.80-2.38)* | 1.89(1.60-2.24)* | 1.79(1.53-2.11)* |
| **Gender** | | | |
| Female | | 1.00 | 1.00 |
| Male | | 1.16(1.02-1.32)* | 1.16(1.02-1.32)* |
| **Education** | | | |
| College Graduate | | 1.00 | 1.00 |
| Some College | | 1.63(1.43-1.86)* | 1.58(1.38-1.82)* |
| High School | | 2.22(1.92-2.55)* | 2.10(1.81-2.42)* |
| <High School | | 2.19(1.81-2.66)* | 2.00(1.63-2.44)* |
| **Race** | | | |
| non-Hispanic white | | 1.00 | 1.00 |
| non-Hispanic black | | 1.97(1.63-2.39)* | 1.97(1.63-2.39)* |
| Mexican American | | 0.74(0.62-0.87)* | 0.73(0.62-0.86)* |
| Other | | 0.94(0.76-1.16) | 0.93(0.75-1.16) |
| **Smoking Status** | | | |
| Never Smoked | | 1.00 | 1.00 |
| Former Smoker | | 1.33(1.15-1.54)* | 1.32(1.14-1.53)* |
| Current Smoker | | 1.67(1.46-1.92)* | 1.59(1.38-1.82)* |
| **Augmented WC** | | | |
| No | | 1.00 | 1.00 |
| Yes | | 1.19(1.03-1.37)* | 1.17(1.01-1.36)* |
| **Physical Activity** | | | |
| Meets Recommendation | | | 1.00 |
| Insufficiently Active | | | 1.10(0.96-1.27) |
| Inactive | | | 1.26(1.07-1.47)* |

Independent variables included in Model 1: C - reactive protein (CRP) and age; Model 2: CRP, age, gender, education (<high school vs. college graduate, high school graduate vs. college graduate, and some college vs. college graduate), race ((non-Hispanic(nH) black vs. nH white, Hispanic vs. nH white, and other vs. nH white)), smoking (current vs. never and former vs. never), and augmented waist circumference(WC) (men ≥ 102 cm, women ≥ 88 cm); Model 3 included all variables from Model 2 and physical activity PA (insufficiently active vs. meeting PA recommendation and inactive vs. meeting PA recommendation).
*Significant predictors ($P$<0.05)
Abbreviations: OR, odds ratio; CI, confidence interval

Age-adjusted analysis revealed significantly increased odds of reporting three or more hours of ST for participants with elevated CRP when compared to a referent group with values ≤3 mg/L (OR 1.37; CI 1.23-1.53, P<0.001). The association between elevated CRP and increased ST remained significant following adjustments for demographic and lifestyle factors (OR 1.27; CI 1.15-1.41, P<0.001) and PA volume (OR 1.25; CI 1.13-1.39, P<0.001). In comparison to a referent group with CRP values ≤3 mg/L, those with values >3 mg/L-10 mg/L, were 25% more likely (P<0.001) to report three or more hours of ST per day, independent of self-reported PA volumes and degree of central adiposity. Furthermore, U.S. adults reporting no PA were 26% more likely (P=0.006) to report three or more hours of ST per day. In comparison to a referent group aged 20-39 years, those ≥60 years of age were approximately two times more likely (P<0.001) to report three or more hours of ST in all three models. In comparison to women, men were significantly (P=0.021) more likely to report three or more hours of ST per day.

Additionally, compared to college graduates and individuals reporting never smoking, education level (P<0.001, all levels) and current smoking status (P<0.001, all levels) reveal a similar increased odds for reporting three or more hours of ST per day. Our study findings revealed mixed results when examining race. In comparison to a referent group of non-Hispanic whites, the odds of reporting three or more hours per day of ST in non-Hispanic blacks was significantly higher (OR1.97; CI 1.63-2.39, P<0.001), while Mexican Americans were found to have significantly lower odds (OR 0.73; CI 0.62-0.86, P<0.001).

**Discussion**

In a nationally representative sample of U.S. adults we found that elevated CRP concentrations were positively associated with self-reporting three or more hours of ST per day. We also found a statistically significant trend (P=0.021) when adjusting for PA volume (Table 1). To our knowledge this is the first study to examine the associations among increased amounts self-reported ST with elevated CRP concentrations serving as the independent variable of interest.

These population based findings add to the current evidence regarding the harmful associations between increased sedentary behavior and CRP (1,12,13,24). In a recent review of prospective studies examining the associations between self-reported ST and sitting time and CVD, Ford et al. (25) reported that greater sedentary time is associated with an increased risk of fatal and non-fatal CVD independent of volume of PA. Other prospective studies have also supported an independent relationship between sedentary time and type II diabetes, in addition to all-cause and CVD mortality (26). However, though many prospective and cross-sectional studies have demonstrated a deleterious association between increased sedentary behavior and CVD, adults continue to engage in increased amounts of ST, with television being the most commonly reported leisure time sedentary activity (6,27,28).

Our findings are consistent with those of Stamatakis et al. (12), who reported that in a sample (n=4,512) of adult (≥35yr) Scottish Health Survey respondents, CRP levels were approximately three fold higher in those spending >4 hours per day in ST. This explained much of the observed ST and CVD association. The observed associations between CRP and ST were consistent across sex, age, and ethnic subgroups.

The findings from sedentary time studies using objectively measured data also support our findings (13). Healy et al. (13) reported that independent of covariates including exercise time, there were statistically significant detrimental associations between sedentary time and CRP in a representative sample of 4,757 adult (≥20 yr) who participated in the 2003-06 NHANES. These investigators reported that sedentary time was detrimentally associated with CRP concentrations, while breaks in sedentary time were beneficially associated.

The findings of this study become increasingly important when considering the implications in the workplace. Sedentary jobs have been reported to have increased 83% since 1950, and Americans work approximately 47 hours a week, which is 164 more hours a year than 20 years ago (29). Since this trend toward increased ST is likely to coincide with technological advances, it will become increasing important to consider new ways to increase overall PA participation (30).

The strengths of our study include strong external validity due to the use of a large representative sample of U.S. adults, and the use of objective measures of inflammation and other CVD risk factors. Our study is not without limitations. First, the cross-sectional study design limits our ability to make causal inferences about the relationship between ST and CRP. Second, the sample analyzed is limited to the non-institutionalized population. Third, self-reported responses were subject to the social desirability effect. Since self-reported estimations are subject to bias, this may have resulted in an under or over estimation of the strength of the associations between the variables of interest. Lastly, though many covariates were considered, other unknown factors may have contributed to the observed associations.

## Conclusions

In this cross-sectional study, elevated levels of CRP were significantly associated with greater amounts of self-reported ST independent of PA or augmented central adiposity. Therefore, decreasing sedentary ST may become an important addition to future recommendations for regular PA. Communicating the importance of reducing ST and increasing PA is becoming an increasingly important goal for health care professionals. Future studies should continue to investigate CRP, objectively measured sedentary behaviors, and the clinical implications for identifying associated cardio-metabolic risk factors.

**References**


1. Jialal I, Devaraj S, Venugopal SK. C-reactive protein: risk marker or mediator in atherothrombosis? Hypertension 2004;44:6-11.
2. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation 2003;107:363-9.
3. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003;107:499-511.
4. Ford ES, Giles WH, Myers GL, Mannino DM. Population distribution of high-sensitivity C-reactive protein among US men: findings from National Health and Nutrition Examination Survey 1999-2000. Clin Chem 2003;49:686-90.
5. Ford ES, Giles WH, Mokdad AH, Myers GL. Distribution and correlates of C-reactive protein concentrations among adult US women. Clin Chem 2004;50:574-81.
6. Nielsen Co. The Cross-Platform Report: How Viewers Watch Time-Shifted Programming [Internet].
7. Grontved A, Hu FB. Television viewing and risk of type 2 diabetes, cardiovascular disease, and all-cause mortality: a meta-analysis. JAMA 2011;305:2448-55.
8. Ford ES. Does exercise reduce inflammation? Physical activity and C-reactive protein among U.S. adults. Epidemiology 2002;13:561-8.

9. Katzmarzyk PT, Lee IM. Sedentary behaviour and life expectancy in the USA: a cause-deleted life table analysis. BMJ Open 2:

10. Sisson SB, Camhi SM, Church TS, et al. Leisure time sedentary behavior, occupational/domestic physical activity, and metabolic syndrome in U.S. men and women. Metab Syndr Relat Disord 2009;7:529-36.

11. Jakes RW, Day NE, Khaw KT, et al. Television viewing and low participation in vigorous recreation are independently associated with obesity and markers of cardiovascular disease risk: EPIC-Norfolk population-based study. Eur J Clin Nutr 2003;57:1089-96.

12. Stamatakis E, Hamer M, Dunstan DW. Screen-based entertainment time, all-cause mortality, and cardiovascular events: population-based study with ongoing mortality and hospital events follow-up. J Am Coll Cardiol 2011;57:292-9.

13. Healy GN, Matthews CE, Dunstan DW, Winkler EA, Owen N. Sedentary time and cardio-metabolic biomarkers in US adults: NHANES 2003-06. Eur Heart J 2011;32:590-7.

14. Ford ES. Body mass index, diabetes, and C-reactive protein among U.S. adults. Diabetes Care 1999;22:1971-7.

15. National Center for Health Statistics Centers for Disease Control and Prevention. NHANES Analytic and Reporting Guidelines. September 2006 version.

16. 2003-2004 Data Documentation, Codebook, and Frequencies. Physical Activity.

17. 2005-2006 Data Documentation, Codebook, and Frequencies. Physical Activity.

18. Laboratory Procedure Manual. C-Reactive Protein in Serum NHANES 2003–2004.

19. Laboratory Procedure Manual. C-Reactive Protein in Serum NHANES 2005–2006.

20. Grundy SM, Cleeman JI, Daniels SR, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 2005;112:2735-52.

21. Haskell WL, Lee IM, Pate RR, et al. Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. Circulation 2007;116:1081-93.

22. SAS. Base SAS 9.2 Procedures Guide. Carey, NC: 2009.

23. Research Triangle Institute. SUDAAN User's Manual, Release 8.0. Research Triangle Park, NC: 2001.

24. Yates T, Khunti K, Wilmot EG, et al. Self-reported sitting time and markers of inflammation, insulin resistance, and adiposity. Am J Prev Med 2012;42:1-7.

25. Ford ES, Caspersen CJ. Sedentary behaviour and cardiovascular disease: a review of prospective studies. Int J Epidemiol 2012;41:1338-53.

26. Proper KI, Singh AS, van Mechelen W, Chinapaw MJ. Sedentary behaviors and health outcomes among adults: a systematic review of prospective studies. Am J Prev Med 2011;40:174-82.

27. Dietz WH. The role of lifestyle in health: the epidemiology and consequences of inactivity. Proc Nutr Soc 1996;55:829-40.

28. Wijndaele K, Brage S, Besson H, et al. Television viewing time independently predicts all-cause and cardiovascular mortality: the EPIC Norfolk study. Int J Epidemiol 2011;40:150-9.

29. The Price of Inactivity. American Heart Association, Inc.: . 2012.

30. Hamilton MT, Hamilton DG, Zderic TW. Role of low energy expenditure and sitting in obesity, metabolic syndrome, type 2 diabetes, and cardiovascular disease. Diabetes 2007;56:2655-67.

# Appendix C

## Associations between Vigorous Intensity Physical Activity and C - reactive Protein in U.S. Adults: NHANES 1999-2006

**Objectives** We sought to examine the associations between high sensitivity C - reactive protein (CRP) and vigorous intensity physical activity (VIPA) in a nationally representative sample of U.S. adults.

**Background** Though evidence has revealed inverse associations between physical activity and CRP, the research on the associations between VIPA and elevated CRP levels using nationally representative samples remains sparse.

**Methods** Sample (n=6,242) included adults (≥20 years of age) that participated in the 1999-2006 National Health and Nutrition Examination Survey. Reported VIPA was categorized into none, insufficient (<500 MET·min·wk$^{-1}$), and meeting the 2008 Department of Health and Human Services PA recommendation (≥500 MET·min·wk$^{-1}$). The dependent variable was elevated CRP (3<CRP≤10 mg/L). Logistic regression analysis was used to estimate odds and adjust for potential confounding variables.

**Results** Analysis revealed significantly lower odds of having elevated CRP for those reporting insufficient volumes of VIPA (OR 0.70; 95% CI 0.58-0.85, P=0.0004) or meeting the 2008 DHHS PA recommendation (OR 0.66; 95% CI 0.53-0.82, P=0.0003). This protective inverse association was independent of several metabolic risk factors.

**Conclusions** In a representative sample of U.S. adults, reporting any VIPA was associated with significantly lower odds of having an elevated CRP level when compared to those reporting no VIPA. These results suggest an

inverse dose-response relationship may exist between VIPA and elevated CRP levels. Future studies should examine the associations among objectively measured VIPA, CRP, and other markers of metabolic health.

C - reactive protein (CRP) is an acute phase marker of inflammation that has shown efficacy in predicting incident cardiovascular disease (CVD) (1). In a statement for healthcare professionals from the Center of Disease Control and Prevention (CDC), and the American Heart Association (AHA), decreased levels of CRP were associated with increased physical activity (PA) and endurance exercise (2). In this statement the recommended diagnostic cut-points for the high-sensitivity CRP assay were as follows; relative risk categories (low, average, high) correspond to approximate values of <1.0, 1.0 to 3.0, and >3.0 mg/L, respectively. It is also recommended that values exceeding 10 mg/L be discarded and other causes of inflammation should be investigated.

The 2008 Department of Health and Human Services (DHHS) PA guidelines for Americans recommend 150 minutes of moderate intensity PA (MIPA) per week or 75 minutes of vigorous intensity PA (VIPA) per week, or an aggregate of both, for overall health benefits and prevention of chronic diseases (3). A recent review of several cross-sectional studies revealed a consistent inverse association between PA and CRP (4). However, overall there is a paucity of research on the associations of VIPA with elevated CRP levels using nationally representative samples. The purpose of this study was to examine the associations between volumes of VIPA and elevated CRP in a nationally representative sample of U.S. adults

**Methods**

This cross-sectional study utilized eight years of data from the 1999-2006 NHANES, a continuous survey conducted by the National Center for Health Statistics (5). The NHANES uses complex, multistage probability design to obtain a representative sample

of the non-institutionalized U.S. population over the age of two months. The total 1999-2006 NHANES sample N was 40,124. Participants in this study met the following inclusion criteria; were ≥20 years of age; provided the necessary VIPA data; provided a serum sample for the measurement of CRP in a mobile examination center; if female, not pregnant; and completed the required informed consent. The final sample that met the study inclusion and exclusion criteria consisted of 6,242 U.S. adults ≥20 years of age.

Four categories of race were used: non-Hispanic white, non-Hispanic black, Mexican American, and other. A three level smoking status variable was created: current smoker, former smoker, and non-smoker. A dichotomous diabetes status variable was created using fasting blood glucose levels (fasting glucose level ≥126 mg/dL, or <126 mg/dL), diabetes medication use, and self-reported diabetes utilizing the following questions from the diabetes questionnaire files within NHANES, DIQ010 *"{Other than during pregnancy, {have you/has SP}/{Have you/Has SP}} ever been told by a doctor or health professional that {you have/{he/she/SP} has} diabetes or sugar diabetes?"* (yes/no) and DIQ070 *"{Is SP/Are you} now taking diabetic pills to lower {{his/her}/your} blood sugar? These are sometimes called oral agents or oral hypoglycemic agents"* (yes/no). A dichotomous low-density lipoprotein cholesterol (LDL-C) variable was created based on the current definition of high levels of LDL-C (<160 mg/dL vs. ≥160 mg/dL) (6). Reduced high density lipoprotein cholesterol (HDL-C) was dichotomized for men (≥40 mg/dL or <40 mg/dL) and women (≥50 mg/dL or <50 mg/dL) and augmented waist circumference (WC) was dichotomized for men (≥102 cm, yes/no) and women (≥88 cm, yes/no) based on National Heart, Lung, and Blood Institute/AHA guidelines (7).

The independent variable of interest in this study was volume of self-reported VIPA. Data from the NHANES individual PA files (8) were accessed to estimate volumes of VIPA. These files include information on different types of PA. The compendium of PA (9) was used to assign metabolic equivalent (MET) levels to each activity. METs are obtained by dividing oxygen uptake in $mL \cdot kg \cdot min^{-1}$ by 3.5 $mL \cdot kg \cdot min^{-1}$ (10). The average duration (minutes per session) was then multiplied by the average frequency (number of sessions per week) and intensity level (METs) to calculate the $MET \cdot min \cdot wk^{-1}$ specific to each VIPA (>6METs). The continuous $MET \cdot min \cdot wk^{-1}$ variable was used to create a three level categorical VIPA variable. The first level represented those reporting no VIPA during the past month (referent). The remaining $MET/min/wk^{-1}$ were categorized as those reporting <500 and $\geq$500 $MET \cdot min \cdot wk^{-1}$ of VIPA. These levels correspond to those not meeting the 2008 DHHS recommendation (3) and those reporting engaging in a level of VIPA meeting the PA recommendation, respectively.

The dependent variable in this study was an elevated serum CRP concentration (3 mg/L<CRP$\leq$10 mg/L). This level corresponds to the high risk CRP level as designated by the statement to healthcare professionals by CDC and the AHA (2). Participant CRP concentrations were measured using latex enhanced nephelometry on a Dade Behring Nephelometer II Analyzer System at the University of Washington Medical Center. Seattle, Washington (11).

Data from the 1999-2006 NHANES were analyzed to determine the odds of having elevated CRP concentrations by level of reported VIPA participation. The data were initially managed using SAS 9.2 (12). SAS was used to conduct complex variable

recodes and data coding validation. SAS-callable SUDAAN (13) was used to conduct the analysis and incorporate the sampling weights within the context of the correlated multi-stage complex sampling design inherent to NHANES. PROC DESCRIPT was used for calculating age-adjusted prevalence estimates of elevated CRP for the categorical variables. Multivariable logistic regression models, using PROC RLOGIST, were created to estimate the odds ratios and 95% confidence intervals for participants who reported any volume of VIPA having elevated CRP. The logistic regression model adjusted for gender, race, smoking status, LDL-C, HDL-C, diabetes, and WC. Any participants who responded ‘don’t know/not sure’, refused to answer, or had missing responses for any of the questions or measures were excluded from the final analysis.

**Results**

Table 1 illustrates the prevalence estimates for elevated CRP according to sample characteristics.

**Table 1. Prevalence of Elevated CRP According to Sample Characteristics: NHANES 1999-2006**

| *Covariates* | | *N* | *Weighted % (SE %)* |
|---|---|---|---|
| **Total** | | 6242 | 29.5 (0.81) |
| **VIPA** | | | |
| | None | 4297 | 33.2 (0.99) |
| | <500 MET·min·wk$^{-1}$ | 873 | 24.1 (1.60) |
| | ≥500 MET·min·wk$^{-1}$ | 1072 | 20.5 (1.58) |
| **Age** | | | |
| | 20-39 | 2107 | 24.0 (1.21) |
| | 40-59 | 1949 | 31.3 (1.41) |
| | ≥60 | 2186 | 36.0 (1.42) |
| **Gender** | | | |
| | Male | 3294 | 25.3 (0.94) |
| | Female | 2948 | 33.8 (1.09) |
| **Race** | | | |
| | non-Hispanic white | 3280 | 28.8 (0.95) |
| | non-Hispanic black | 1145 | 32.0 (1.50) |
| | Mexican American | 1370 | 36.2 (1.35) |
| | Other | 447 | 27.6 (2.99) |
| **Smoking Status** | | | |
| | Never Smoked | 3175 | 27.5 (0.96) |
| | Former Smoker | 1711 | 28.7 (1.93) |
| | Current Smoker | 1356 | 35.0 (1.58) |
| **LDL-C (mg/dL)** | | | |
| | <160 | 5440 | 28.8 (0.74) |
| | ≥160 | 802 | 34.4 (2.57) |
| **HDL-C (mg/dL)** | | | |
| | ≥40, men; ≥50, women | 4475 | 25.3 (0.89) |
| | <40, men; <50, women | 1767 | 40.1 (1.77) |
| **Diabetes** | | | |
| | No | 5569 | 28.6 (0.85) |
| | Yes | 673 | 46.1 (3.89) |
| **Augmented WC** | | | |
| | No | 3146 | 17.8 (0.85) |
| | Yes | 3096 | 43.6 (1.13) |

Independent variables included vigorous intensity physical activity (VIPA), age (years), gender, race, smoking status, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), diabetes status, and augmented waist circumference (WC) men (yes: ≥102 cm, no: <102 cm), women (yes: ≥88 cm, no <88 cm).
Abbreviations: Elevated CRP, elevated C - reactive protein (>3 mg/L to ≤10 mg/L)

Age-adjusted prevalence estimate of elevated CRP concentration among U.S. adults meeting the study inclusion criteria was 29.5%. Prevalence estimates of elevated CRP increased with age (20-39 [24.0%], 40-59 [31.3%], and ≥60 years of age [36%]) and were found to be greater among women (33.8%), Mexican-Americans (36.2%) and non-Hispanic blacks (32.0%). Prevalence estimates of elevated CRP were found to be higher in former (28.7%) and current smokers (35.0%) as well as participants with high LDL-C

(34.4%) and reduced HDL-C (40.1%). Prevalence estimates of elevated CRP among individuals according to diabetes status was 46.1% and 28.6% for those classified as having diabetes and not having diabetes, respectively. Prevalence estimates of elevated CRP increased with central adiposity, 43.6% for those classified as having an augmented WC compared to 17.8% for those not classified with greater levels of central adiposity.

Table 2 shows the results of the logistic regression analysis examining the associations between elevated CRP and the independent variables.

**Table 2. Elevated C - reactive Protein and Independent Variables: NHANES 1999-2006**

| Odds of Having an Elevated CRP Level | | |
|---|---|---|
| *Variable* | *OR (95% CI)* | *P-Value* |
| **VIPA** | | |
| None | 1.00 | |
| <500 MET·min·wk$^{-1}$ | 0.70(0.58-0.85)* | <0.001 |
| ≥500 MET·min·wk$^{-1}$ | 0.66(0.53-0.82)* | <0.001 |
| **Gender** | | |
| Male | 1.00 | |
| Female | 1.32(1.16-1.50)* | <0.001 |
| **Race** | | |
| non-Hispanic white | 1.00 | |
| non-Hispanic black | 1.08(0.91-1.29)* | 0.375 |
| Mexican American | 1.32(1.12-1.56) | 0.001 |
| Other | 0.96(0.72-1.28) | 0.794 |
| **Smoking Status** | | |
| Never Smoked | 1.00 | |
| Former Smoker | 1.19(0.98-1.45) | 0.081 |
| Current Smoker | 1.47(1.21-1.77)* | <0.001 |
| **LDL-C (mg/dL)** | | |
| <160 | 1.00 | |
| ≥160 | 1.26(1.02-1.55)* | 0.036 |
| **HDL-C (mg/dL)** | | |
| ≥40, men; ≥50, women | 1.00 | |
| <40, men; <50, women | 1.43(1.18-1.73)* | <0.001 |
| **Diabetes** | | |
| No | 1.00 | |
| Yes | 1.25(1.01-1.55)* | 0.037 |
| **Augmented WC** | | |
| No | 1.00 | |
| Yes | 3.29(2.86-3.80)* | <0.001 |

Independent variables included vigorous intensity physical activity (VIPA) (< 500 MET·min·wk$^{-1}$ vs. none and ≥500 MET·min·wk$^{-1}$ vs. none), gender (female vs. male), race ((non-Hispanic (nH) Black vs. nH White, Hispanic vs. nH White, and other vs. nH White)), smoking status(current vs. never and former vs. never), low-density lipoprotein cholesterol ≥160 mg/dL (yes vs. no), high-density lipoprotein cholesterol men (≥40 mg/dL, yes vs. no), women (≥50 mg/dL, yes vs. no), diabetes (yes vs. no) and augmented waist circumference (WC) men (yes: ≥102 cm vs. no: <102 cm), women (yes: ≥88 cm vs. no: <88 cm).
*Significant predictors ($P$<0.05)
Abbreviations: Elevated CRP, C - reactive protein (>3 mg/L to ≤10 mg/L); OR, odds ratio; CI, confidence interval; MET, metabolic equivalent

In our initial logistic regression, a statistically non-significant increase in odds of having elevated CRP was observed in those 40-59 yr (P=0.220) and those ≥60 yr (P=0.118) when compared to those 20-39 yr (referent group). Moreover, a statistically significant relationship between age and elevated CRP was not revealed (P=0.257). Thus, the final analysis does not include age as a covariate. Final analysis revealed significantly

lower odds of having elevated CRP for subjects reporting insufficient VIPA (OR 0.70; 95% CI 0.58-0.85, P=0.0004) or volumes of VIPA meeting the 2008 DHHS recommendation (OR 0.66; 95% CI 0.53-0.82, P=0.0003). This protective inverse association was independent of several metabolic risk factors, including; having an augmented WC, a high LDL-C level, a reduced HDL-C level, diabetes, and smoking. In comparison to a referent group of male participants, the odds of having an elevated CRP level were 32% higher in female survey participants (OR 1.32; 95% CI 1.16-1.50, P<0.001). When examining race with non-Hispanic whites serving as our referent, the odds of having elevated CRP levels were 32% higher non-Mexican Americans (OR 1.32; 95% CI 1.12-1.56, P=0.001), while an increased odds of having elevated CRP in non-Hispanic blacks was not statistically significant (P=0.375). The odds of having an elevated CRP level were 47% higher for current smokers when compared to a referent group reporting never smoking (P<0.001). This analysis also revealed significantly higher odds of having elevated CRP for participants with LDL-C concentrations ≥160 mg/dL (OR 1.26; 95% CI 1.02-1.55, P=0.036) or reduced HDL-C concentrations (OR 1.43; 95% CI 1.18-1.73, P<0.001). The odds of those classified as having diabetes having elevated CRP were significantly higher (OR 1.25; 95% CI 1.01-1.55, P=0.037) when compared to those classified as not having diabetes. Lastly, the odds of those with an augmented WC having an elevated CRP level were approximately 3.3 times higher (OR 3.29; 95% CI 2.86-3.80, P<0.001) compared to a referent group of survey participants not classified as having an augmented WC.

**Discussion**

The current PA guidelines for U.S. adults (3) recommend a minimum of 150 minutes of MIPA or 75 minutes of VIPA per week. In this nationally representative sample of U.S. adults, reporting any volume ($MET \cdot min \cdot wk^{-1}$) of VIPA was associated with a significant decrease in odds of having an elevated CRP level. Furthermore, our findings illustrate a statistically significant inverse dose-response relationship between reported VIPA and having an elevated CRP level. Interestingly, our analysis revealed significantly lower odds of having an elevated CRP level for those reporting volumes of VIPA that met the 2008 DHHS recommendation as well as for those reporting insufficient volumes. In contrast to the findings of other cross-sectional analyses that investigated high levels of physical activity participation (min/wk) (14,15), these associations remained statistically significant following adjustments for adiposity.

Our findings add to the current evidence demonstrating an inverse relationship between PA and CRP in U.S. adults (16,17) independent of other major risk factors (2). In an analysis of 13,748 adult participants of the Third NHANES, reporting vigorous leisure-time physical activity was associated with 47% lower odds of having an elevated CRP level when compared to a sedentary referent group (16). Inverse associations between activities of vigorous intensity and CRP have also been reported when comparing the CRP levels of non- and infrequent exercisers (0 to 11 times/mo) to those who engage in regular exercise (≥12 times/mo) (17). Among 4,072 adult participants in the Third NHANES, analysis by King et al.(17) revealed significantly ($P<0.05$) lower likelihood of elevated CRP among those reporting regular participation in jogging, swimming, cycling, aerobic dancing, calisthenics, and weight lifting when using non and

infrequent exercisers (0-11 times/mo) as the referent group. Following adjustment for age, race or ethnicity, gender, body mass index, smoking, and health status, the association remained significant in those reporting jogging (OR 0.33; 95% CI 0.14–0.78, P<0.05) or aerobic dancing (OR 0.31; 95% CI 0.13–0.78, P<0.05) 12 or more times/mo when compared to a referent group of non and infrequent exercisers. The results of the aforementioned studies, as well as those from our study, suggest that the observed reduction in odds of having elevated CRP may be associated with the intensity of PA. Furthermore, recent research using data from the 1999-2004 NHANES has revealed a significant (P<0.01) inverse dose-response relationship between VIPA and metabolic syndrome (18), which is known to be associated with elevated CRP levels (19).

Our findings also support those reported in prospective studies revealing statistically significant reductions in CRP levels for increased levels of moderate to vigorous PA participation. In a 12 week study that implemented a combination of aerobic and resistance exercise a 58% reduction in CRP levels was reported (20). Similarly, investigators have reported a 60% reduction in the CRP levels of participants in a 15-week trial involving a combination of endurance and resistance exercise (21). The current findings add to previous work and illustrate the importance of examining the relationships between PA, the volume and intensity of the PA, and other factors that are associated with CRP levels.

The strengths of this study include the use of a large representative sample of U.S. adults and the use of objectively measured markers of cardio-metabolic risk. The limitations include those inherent to utilization of cross-sectional data, which does not allow the ability to make causal inferences about the relationship between VIPA and

CRP. The estimations of VIPA participation were derived from self-report data, which may have bias due to social desirability effect and recall. These factors may have resulted in the under or over estimation of the reported associations.

## Conclusions

Our findings suggest that a statistically significant inverse dose-response relationship exists between self-reported volumes of VIPA and having an elevated CRP level. These results also suggest that this relationship is independent of central adiposity, a known mediator in the relationship between PA and CRP. Future studies should examine the associations among objectively measured VIPA and elevated CRP levels.

**References**


1. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation 2003;107:363-9.
2. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003;107:499-511.
3. Physical activity guidelines for Americans; Be active, healthy, and happy. In: Services USDoHaH, ed., 2008.
4. Kasapis C, Thompson PD. The effects of physical activity on serum C-reactive protein and inflammatory markers: a systematic review. J Am Coll Cardiol 2005;45:1563-9.
5. National Center for Health Statistics Centers for Disease Control and Prevention. NHANES Analytic and Reporting Guidelines. September 2006 version.
6. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA 2001;285:2486-97.
7. Grundy SM, Cleeman JI, Daniels SR, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 2005;112:2735-52.
8. CDC. Centers for Disease Control and Prevention. Codebook for data collection; Physical Activity Section of the SP and MEC CAPI Questionnaire.

9. Ainsworth BE, Haskell WL, Whitt MC, et al. Compendium of physical activities: an update of activity codes and MET intensities. Med Sci Sports Exerc 2000;32:S498-504.

10. Howley ET. Type of activity: resistance, aerobic and leisure versus occupational physical activity. Med Sci Sports Exerc 2001;33:S364-9; discussion S419-20.

11. Laboratory Procedure Manual. C-Reactive Protein in Serum NHANES 2005–2006.

12. SAS. Base SAS 9.2 Procedures Guide. Carey, NC: 2009.

13. Research Triangle Institute. SUDAAN User's Manual, Release 8.0. Research Triangle Park, NC: 2001.

14. Colbert LH, Visser M, Simonsick EM, et al. Physical activity, exercise, and inflammatory markers in older adults: findings from the Health, Aging and Body Composition Study. J Am Geriatr Soc 2004;52:1098-104.

15. Verdaet D, Dendale P, De Bacquer D, Delanghe J, Block P, De Backer G. Association between leisure time physical activity and markers of chronic inflammation related to coronary heart disease. Atherosclerosis 2004;176:303-10.

16. Ford ES. Does exercise reduce inflammation? Physical activity and C-reactive protein among U.S. adults. Epidemiology 2002;13:561-8.

17. King DE, Carek P, Mainous AG, 3rd, Pearson WS. Inflammatory markers and exercise: differences related to exercise type. Med Sci Sports Exerc 2003;35:575-81.

18. Churilla JR, Fitzhugh EC. Total physical activity volume, physical activity intensity, and metabolic syndrome: 1999-2004 National Health and Nutrition Examination Survey. Metab Syndr Relat Disord 2012;10:70-6.

19. Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. Circulation 2003;107:391-7.

20. Stewart LK, Flynn MG, Campbell WW, et al. The influence of exercise training on inflammatory cytokines and C-reactive protein. Med Sci Sports Exerc 2007;39:1714-9.

21. Daray LA, Henagan TM, Zanovec M, et al. Endurance and resistance training lowers C-reactive protein in young, healthy females. Appl Physiol Nutr Metab 2011;36:660-70.

# Vita

Michael Ryan Richardson is a graduate research assistant and instructor in the Clinical and Applied Movement Sciences department at the University of North Florida in Jacksonville, Florida. He also completed his undergraduate coursework at the University of North Florida where he earned his Bachelors of Science degree in Exercise Science. He began his career in the medical field as a Surgical Technologist at Baptist Medical Center-Nassau in 2002 after he successfully completed the Surgical Technologist program at Florida State College at Jacksonville.

His research studies have included the investigation of the associations between physical activities, classified as both moderate or vigorous, inactivity, and various markers of cardio-metabolic health. His primary focus has been the use of C - reactive protein as a marker of inflammation. He is married to Kim Richardson. They currently reside in Fernandina Beach, Florida with their two children Alora and Avery.